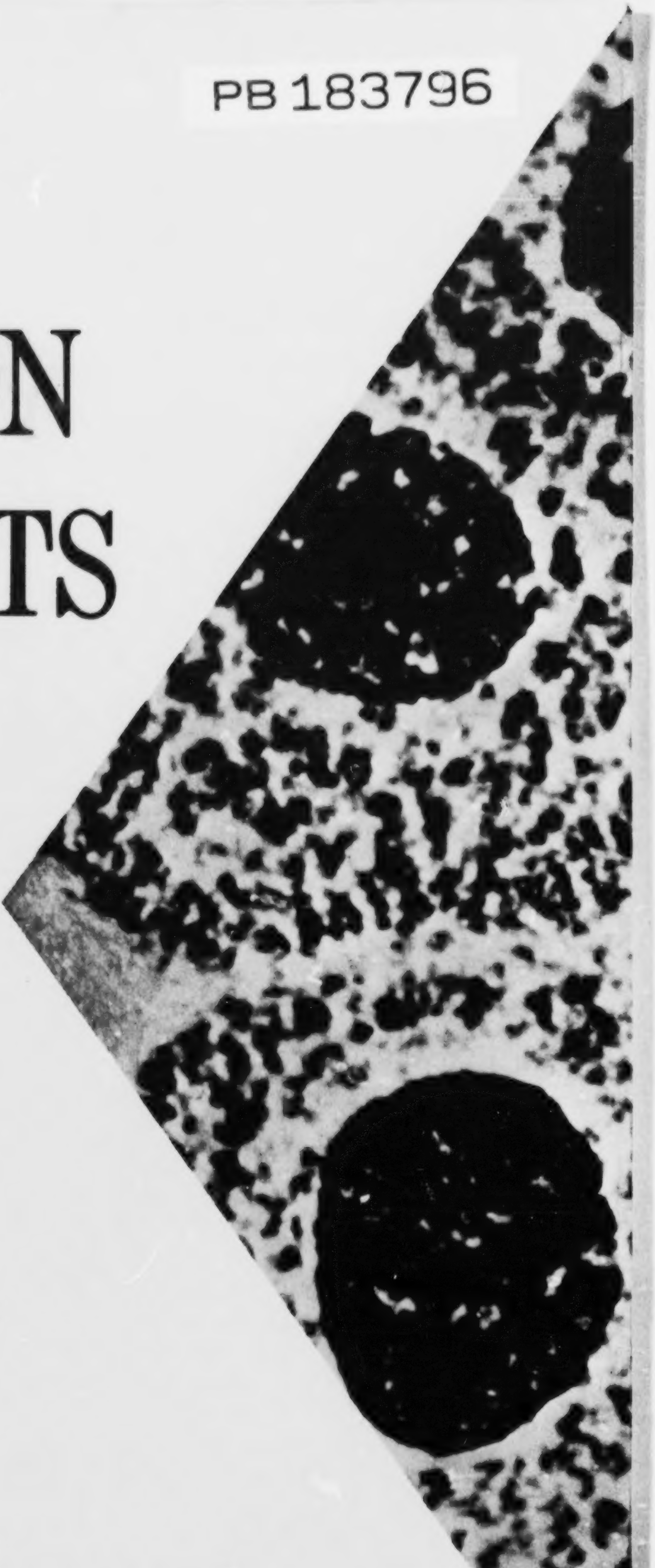
PB 183796

RADIATION BIO-EFFECTS

SUMMARY REPORT

January-December 1967



U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
Public Health Service

Reproduced by the
CLEARINGHOUSE
for Federal Scientific & Technical
Information Springfield Va. 22151

RADIATION BIO-EFFECTS

Summary Report January-December 1967

Dr. A. H. Wolff, Chief
Radiation Bio-Effects Program
Rockville, Maryland 20852

Compiled and Edited by
Donald M. Hodge

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of Disease Prevention and Environmental Control
National Center for Radiological Health

Disclaimer

The use of commercial preparations or products specified in this report in no way constitutes endorsement by the U. S. Public Health Service.

PREFACE

This Second Annual Report summarizes the activities and progress of the Radiation Bio-Effects Program, National Center for Radiological Health, for calendar year 1967. The first section illustrates the organization of the Radiation Bio-Effects Program within the Center and describes the Program Laboratories. The second part discusses specific research conducted within each of the Laboratories. The appendixes list the technical and professional staff of the RBE Program, papers and presentations authored by Program scientists, and titles of seminars and colloquia held at the Rockville, Maryland, Laboratory during the year. The Program gratefully acknowledges the outstanding guidance provided by its scientific advisory committees whose names are listed in the appendix.

Arthur H Wolff

Arthur H. Wolff, Chief
Radiation Bio-Effects Program
National Center for Radiological Health

BLANK PAGE

CONTENTS

Preface.. iii

Radiation Bio-Effects Program.............................. 1

Radiation Bio-Effects Research Summary..................... 5

I. Physiology and Biophysics Laboratory.................... 5
   A. Effect of Whole-Body and Partial-Body X Irradiation on the Induced Synthesis of Tryptophan Pyrrolase..... 6
   B. Relative Rates of Tryptophan Pyrrolase Degradation Following the Administration of Hydrocortisone and Tryptophan to Intact Rats........................... 10
   C. Radiation Effects on Protein Synthesis in the Cell-Free System.. 14
   D. Biochemical Effects in Rats Irradiated *In Utero*...... 15
   E. Radiation Effects on the Structure of Globular Proteins in Solution................................ 16
   F. Further Investigations of the Influence of Glucan on Irradiated and Nonirradiated Mice.................. 17
   G. Isolation of Kupffer Cells from Rat Liver........... 22

II. Radionuclide Toxicology Laboratory..................... 25
   A. Bio-Effects Studies................................. 25
   B. Radiopharmaceutical Metabolism Studies.............. 36
   C. Metabolism and Assessment of Heavy Radionuclides..... 42
   D. Polonium in Tobacco and Tobacco Smoke............... 46

III. Radiation Oncology and Pathology Laboratory........... 47
   A. Radiation-Ethionine Ultrastructure Studies.......... 47
   B. Thyroid Tumorigenesis Studies....................... 52
   C. Host-Virus Relationships in Irradiated Cells......... 53
   D. Radiation-Ethionine Co-Carcinogenesis Study.......... 54
   E. Biochemical Assays of Irradiated Cells.............. 61

IV. Radiation Physics Laboratory........................... 63
   A. Laboratory Services................................. 63
   B. Spectra and Percentage Depth Dose of X Radiation Emitted by a Color Television Receiver.............. 71
   C. Radiological Aspects of X-Ray Epilation in the Treatment of Tinea Capitis............................ 77

V. Radiation Cytology Laboratory........................... 87
   A. Human Studies....................................... 88
   B. Animal Studies...................................... 92

VI. Collaborative Radiological Health Laboratory............ 99
  A. The Barrier Maintained Colony........................ 99
  B. The Long-Term Experiment--Design and Feasibility Studies.. 102
  C. Age Changes.. 104
  D. Irradiation of the Embryo, Fetus and Neonate......... 105
  E. Data Processing.. 106

APPENDIXES

A. Publications January - December 1967............... 107
B. Presentations January - December 1967.............. 111
C. Advisory Committees................................ 114
D. Seminars and Colloquia, 1967....................... 116
E. Technical and Professional Staff................... 119

FIGURES

1. Organization of the Radiation Bio-Effects Program.......... 1
2. Induction Curves Following Hydrocortisone Administration and Whole-Body Irradiation.................................... 8
3. Induction Curves Following Hydrocortisone Administration and Head Only Irradiation..................................... 9
4. Induction Curves Following Hydrocortisone Administration and Body Only (Head Shielded) Irradiation.................. 9
5. Results of Administering Puromycin to Rats Following Tryptophan Pyrrolase Induction by Hydrocortisone........... 11
6. Disappearance of Enzyme Activity After Puromycin Administration.. 12
7. ^{14}C-Phenylalanine Incorporation by Irradiated Rats......... 14
8. Tissue Distribution of ^{14}C-Labeled Glucan................. 18
9. Tissue Distribution of Nonground Mixture of Commercial and ^{14}C-Labeled Glucan...................................... 19
10. Tissue Distribution of Ground Mixture of Commercial and ^{14}C-Labeled Glucan...................................... 19
11. Differences in Physical Size and Dispersion Properties in Nonground and Ground Glucan.............................. 20
12. Electron Photomicrograph of an Unstained Kupffer Cell Containing Carbon Particles.............................. 23
13. Oxygen Uptake of Various Liver Cell Suspensions........... 24
14. Strontium-89 and -90 Uptake Curves Calculated from Strontium-85 Data... 26
15. Strontium-85 Uptake in Felines on Two Diets............... 28

16. Autoradiograph and Dose Contour Map of Feline Femur Labeled with Strontium 90 from Conception........................ 30
17. Densitometric Separation of Tooth Fractions from Felines... 31
18. Iodine-131 Retention in Guinea Pigs....................... 33
19. Weights and Iodine-131 Activity in Embryonic Guinea Pigs... 33
20. Short-Term Retention of Strontium 85 and Calcium 47 in Cats Fed Sodium or Calcium Alginate........................... 37
21. Effect of Sodium Alginate on Strontium-85 Body Burden in Cats.. 37
22. Effect of Alginate in Maternal Diet on Uptake of Strontium 85 in Neonatal Cats...................................... 38
23. Retention of Iodine-131 Labeled Tetracycline in Felines.... 39
24. Excretion of Iodine-131 Labeled Tetracycline Intravenously Injected in Cats............................ 40
25. Nucleus from Hepatoma Cell................................ 49
26. Higher Magnification of Area in Figure 25 Showing Cytoplasmic Remnants and Invagination of the Nuclear Membrane.. 50
27. Part of a Nucleus from a Hepatoma Cell Showing the Invagination of the Nuclear Membrane in What Seems to be the Final Stages of Endocytosis by the Nucleus............ 51
28. Cause of Death in Irradiated Rats Fed Ethionine........... 54
29. Mortality Rate for Each Treatment Group.................. 55
30. Section of Liver from Rat Fed 0.16% Ethionine for 18 Months.. 56
31. Section of Liver from Rat Fed 0.08% Ethionine for 18 Months.. 57
32. Section of Pancreas from a Rat Fed 0.16% Ethionine Showing Parenchymal Atrophy with Fatty Replacement................ 58
33. Relationship Between Radiation, Ethionine, and Incidence of Mammary Tumors... 60
34. Small Animal Irradiation Chamber......................... 65
35. Electrical Device Used to Sacrifice Laboratory Rodents..... 66
36. Schematic Drawings of Aural Rate Meter and Analog-To-Digital Converter.. 68
37. Spectra of X Rays from Shunt Regulator (6EF4) and High Voltage Rectifier (3CN3) Tubes............................ 72
38. Spectra of X Rays from High Voltage Rectifier Tube (3CN3) and X-Ray Machine... 73
39. Spectra of X Rays from Shunt Regulator Tube (6EF4) and X-Ray Machine.. 74
40. Tissue Equivalent Chamber Used in Depth Dose Studies....... 75
41. Central Axis Depth Dose Curves............................ 76
42. Absorption of Brain, Bone, Water and LiF at Various Energies 78
43. Absorption Coefficient of Elektron Metal.................. 79
44. Average Doses in Brain, Eye, and Thyroid for 3- and 6-Year-Old Skull Phantoms....................................... 80
45. Immobilization of the Beagle During X-Ray Exposure of Brain 82

46. Perivascular Lesion in the Forebrain........................ 83
47. Distribution of Mammary Tumors by Percentage of Fat......... 85
48. Electron Photomicrographs of Human Chromosomes............. 90
49. Association Between Sertoli Cells and Germ Line Cells....... 96
50. Aerial View of CRHL Beagle Colony, Fort Collins, Colorado... 100

TABLES

1. Rate Constants for Degradation of Tryptophan Pyrrolase During Hormonal and Substrate Induction of the Enzyme....... 13
2. Initial Rate of Carbon Disappearance....................... 20
3. Microliters of Oxygen Taken Up per Milligram of Dry Tissue per Hour... 24
4. Ca^{++}, Sr^{++}, and Pi in Diet and Bone of Felines............. 28
5. Mean Concentration of ^{210}Po and ^{210}Pb in Human Soft Tissues. 43
6. ^{210}Po, ^{210}Pb, and ^{137}Cs in Reindeer Muscle and Rumen Content from Nunivak Island, Alaska........................ 44
7. Tissue Distribution of Lead 210 and Polonium 210 in Cats.... 45
8. Thyroid Dimensions of Rats at Different Ages................ 52
9. Tumor Incidence Based on 279 Rats in Study................. 60
10. X Irradiations at the Twinbrook Laboratory, 1967............ 63
11. Lipid Components in Rat Mammary Tumors..................... 85
12. Cells with Chromosome Aberrations in a Human Leukemia Patient.. 89

RADIATION BIO-EFFECTS PROGRAM

The National Center for Radiological Health became one of the "Centers" within the Bureau of Disease Prevention and Environmental Control following the reorganization of the U.S. Public Health Service in January 1967. The new Center was organized mainly from its predecessor, the Division of Radiological Health, and with few exceptions, the Center Programs have been carried over from the parent organization.

The Radiation Bio-Effects Program (Figure 1) complements all of the other Center Programs by providing research support on the biological effects of ionizing radiation. Such support includes an interdisciplinary biomedical research program to determine whether radiation levels considered to be of public health significance are deleterious to the exposed populations. Also involved are (1) research on dose-effect phenomena, (2) the development of indixes of radiation damage in mammalian systems which can be used as factors in developing radiation exposure standards, understanding long-term effects, and interpreting epidemiologic studies, and (3) research to identify specific changes induced by radiation which may be useful for detecting or interpreting biological damage from low-level radiation exposures.

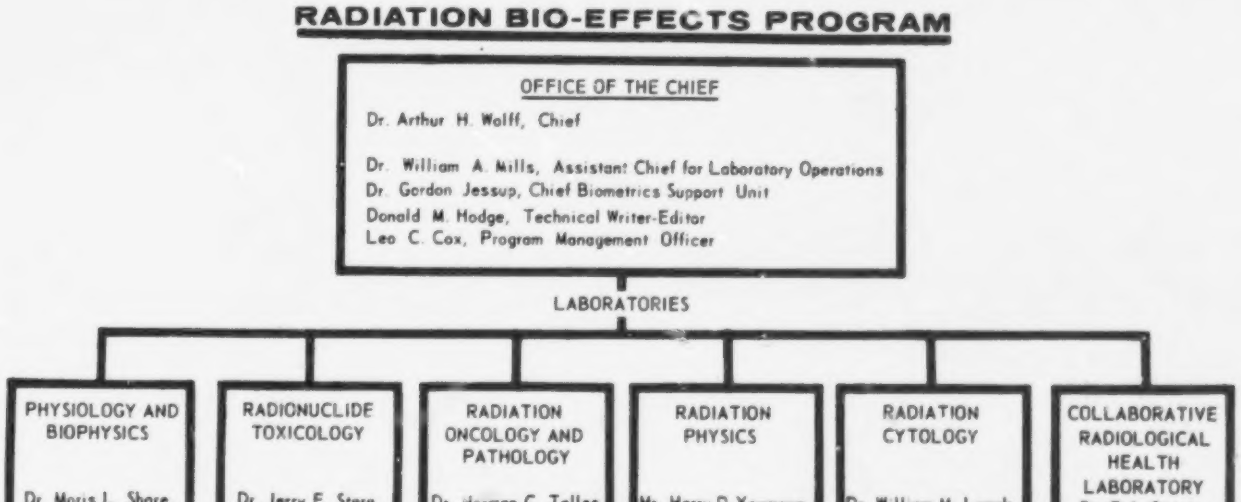

RADIATION BIO-EFFECTS PROGRAM
OFFICE OF THE CHIEF
Dr. Arthur H. Wolff, Chief
Dr. William A. Mills, Assistant Chief for Laboratory Operations
Dr. Gordon Jessup, Chief Biometrics Support Unit
Donald M. Hodge, Technical Writer-Editor
Leo C. Cox, Program Management Officer
LABORATORIES
PHYSIOLOGY AND BIOPHYSICS
Dr. Moris L. Shore, Chief
RADIONUCLIDE TOXICOLOGY
Dr. Jerry F. Stara, Chief
RADIATION ONCOLOGY AND PATHOLOGY
Dr. Norman C. Telles, Chief
RADIATION PHYSICS
Mr. Harry D. Youmans, Chief
RADIATION CYTOLOGY
Dr. William M. Leach, Chief
COLLABORATIVE RADIOLOGICAL HEALTH LABORATORY
Dr. R.J. Garner, Director

Figure 1. Organization of the Radiation Bio-Effects Program.

The use of X rays in diagnostic, therapeutic, and industrial applications is increasing, thereby potentially increasing the exposure to the population. Radiations emitted from electronic products may prove to be an added source of exposure to large numbers of people. Furthermore the increased use of radionuclides for medical and research purposes and the increased use of nuclear energy for peaceful purposes are sure to increase the levels of exposure and the number of persons potentially exposed. We must, therefore, define as nearly as possible the biological effects that are most likely to result from exposures to low doses. Although considerable information exists on the result of exposure to massive radiation doses, we know little of the low-dose effects. The Radiation Bio-Effects Program through its varied research efforts attempts to reveal and understand these effects.

To achieve this objective, the Radiation Bio-Effects Program is divided into several mission-oriented Laboratories. With the exception of the Collaborative Radiological Health Laboratory, Fort Collins, Colorado, and the Radionuclide Toxicology Laboratory, Cincinnati, Ohio, all are located in Rockville, Maryland. Some studies are conducted at the Southwestern Radiological Health Laboratory, Las Vegas, Nevada, or as the need arises, at other NCRH laboratories.

Physiology and Biophysics Laboratory

Using techniques of molecular biology, biochemistry, and physiology, the Physiology and Biophysics Laboratory examines the effects of ionizing radiation on living organisms at the molecular, cellular, and the organ levels. The group devotes particular attention to specific changes induced by radiation in DNA (deoxyribonucleic acid), the consequences of such changes on the synthesis of RNA (ribonucleic acid) and proteins, and the impact of alterations in these macromolecules on physiological function. Primarily the investigators are interested in the long-term effects of ionizing radiation; for example, the sensitivity of animals to radiation at various ages, the amplification or reduction of biological damage with time, and the development of sensitive indixes of radiation damage in mammalian systems. Ancillary studies having short-term objectives are conducted to aid in the interpretation of projected latent effects.

Radionuclide Toxicology Laboratory

The Radionuclide Toxicology Laboratory in Cincinnati deals primarily with the effects of radionuclides on biological systems with emphasis on the radionuclides of strontium and iodine. The group studies the kinetics, pharmacology, and metabolism of specific nuclides, calculates dose following radionuclide administration, and relates the radiation doses from radionuclides to damage in critical organ systems. Particular attention is devoted to the long-term pathological effects on mammals after exposure to low levels of radionuclides. Relationships between the critical times of biological development and the sensitivity to radionuclide deposition are examined. The effectiveness of drugs administered to animals to reduce radionuclide body burdens is evaluated. This Laboratory also supports studies in nuclear medicine, environmental surveillance, and occupational health, and examines the distribution, deposition, and excretion of natural and induced radionuclides in occupational exposure situations.

Radiation Oncology and Pathology Laboratory

The Radiation Oncology and Pathology Laboratory conducts studies on the fundamental nature of radiation carcinogenesis. The principal effort of this research centers on a morphological evaluation of the changes that occur in cells as they become transformed from benign to malignant cells. The approaches include studies of radiation as a carcinogen, and various synergistic and cocarcinogenic factors using techniques of light and electron microscopy, tumor transplantation, changes in the immune response of irradiated tissues, and the transformation of cultured cells. These studies seek information on the role of radiation as a direct carcinogenic agent and what other factors are involved in the production of tumors. This Laboratory also extends its pathology and electron microscopic services to the other Program Laboratories.

Radiation Physics Laboratory

Activities in the Radiation Physics Laboratory are divided between radiological service to the Radiation Bio-Effects Program and research in radiation dosimetry. The radiological service consists of (1) maintaining radionuclide preparation and counting facilities and an irradiation room for X- and γ-ray exposures, and (2) providing technical guidance in dosimetry and counting procedures to radiobiological investigators. The group also conducts concurrent research in dosimetry to correlate the various internal and external radiation exposures which are required in biological investigations. Research to determine dosimetric parameters in epidemiology is conducted for the Population Studies Program.

Radiation Cytology Laboratory

Through both animal and human studies, the Radiation Cytology Laboratory seeks information for assessing acute and persistent cellular damage following exposure to ionizing irradiation. The laboratory group places primary emphasis upon chromosomal changes associated with low-level exposure to X rays and biologically significant radionuclides. The effect upon both somatic and germinal cells and the significance of cytogenetic changes provides an excellent opportunity for furthering knowledge of latent changes in somatic cells and of possible genetic damage.

Collaborative Radiological Health Laboratory

Through a U.S. Public Health Service contract, the Radiation Bio-Effects Program--in collaboration with Colorado State University, Fort Collins--investigates, by inference, the possible effects of irradiation in man be examining and interpreting the biological effects in the beagle which have resulted from known radiation exposures. The studies at the Collaborative Radiological Health Laboratory (CRHL) encompass various aspects of a single whole-body radiation exposure given at various ages including *in utero*.

The long-term studies are designed to determine whether exposures approaching the levels used in diagnostic radiology may be hazardous to man. Nearly 1,700 beagles are scheduled for the long-term study. The investigations have begun to yield information on developmental changes, acute injury, recovery, latent period, and long-term behavioral, systemic, and morphological effects as a function of dose and age at the time of exposure.

RADIATION BIO-EFFECTS RESEARCH SUMMARY

I. PHYSIOLOGY AND BIOPHYSICS LABORATORY

M. L. Shore, Chief

In evaluating the hazards of ionizing radiation, a number of approaches have been taken by the Physiology and Biophysics Laboratory. These are generally based on the hypothesis that ionizing radiation produces its most significant long-term effects through alterations in DNA, the structural integrity of which is essential to the maintenance of all normal cellular activity and thus the functional viability of the living organism. Any radiation-induced alteration, produced directly or indirectly in the normal base sequence of DNA, constitutes a mutation, which may or may not be immediately expressed. Such mutations are generally considered to be deleterious, and, whether expressed or not, constitute damage produced by ionizing radiation. Mutations may find obvious expression, such as radiation-induced birth abnormalities, decreased lifespan, tumor induction, and cataract formation. These effects may become apparent after a variable latent period. Mutations may also find more subtle expression in decreased functional capacity of specific organ systems, alterations in immunological competency that may decrease resistance to bacterial or viral infection (including possible viruses that may play a role in tumorigenesis), and alterations in the synthesis of proteins whose synthesis is directly dependent on the integrity of DNA, messenger RNA, and the translation process.

Present studies examine the production by radiation of somatic mutations which may have a deleterious effect during the life of the irradiated organism. We are particularly concerned with both the effects of radiation delivered in utero, and damage produced by radiation delivered to living organisms after birth.

While the primary interest of the program relates to effects of low levels of irradiation, in some studies relatively high doses of radiation have been employed. These higher doses permit the clear and economical demonstration of radiation effects, and elucidate the biological processes that are affected by radiation. It is most probable that the nature of the damage from high and low doses of radiation is the same. The principal difference will be in the quantity of damage produced. It is recognized, however, that repair of radiation damage may be a factor that will reduce the apparent biological effectiveness of radiations delivered at low doses and low dose rates, although it is also possible that not all radiation damage is subject to repair.

The studies conducted by the Physiology and Biophysics Laboratory investigate changes that may be produced by radiation, which may be subsequently correlated with radiation-induced pathological conditions. They may hopefully contribute to the assessment of radiation damage prior to the time at which irreversible commitment to pathological processes has occurred, and may permit the evaluation of techniques designed to protect or reverse the deleterious effects of ionizing radiation. They will also assist in the development of realistic standards of radiation exposure based on experimental evidence which considers not only dose, but dose rate and energy of the radiation.

A. EFFECT OF WHOLE-BODY AND PARTIAL-BODY X IRRADIATION ON THE INDUCED SYNTHESIS OF TRYPTOPHAN PYRROLASE

E. P. Sheppard, M. L. Shore

A number of investigators have demonstrated that radiation interacts with DNA to produce cross-linkage of DNA strands, base changes, and degradation of DNA in cells. What these radiation-induced alterations in DNA structure mean, however, in terms of the ability of resting, nondividing, mammalian cells to function normally, is not well understood. From the observed effects of radiation on DNA structure, it might reasonably be inferred that the process of DNA transcription may be changed by ionizing radiation. Since DNA transcription leads to synthesis of proteins required for normal cell function, an effect of irradiation on this process would be reflected in changes in protein synthesis in the irradiated organism.

Harrington's work is suggestive of an effect of radiation on DNA transcription[1]. She showed that the priming activity of DNA for RNA polymerase was depressed by X irradiation. Pollard's work[2] with *E. coli* demonstrated that the induction of β-galactosidase was blocked by radiation, indicative of a similar effect of radiation on the process of transcription.

Previously published work from our laboratory using rats irradiated *in utero* demonstrated that radiation interferes with the subsequent normal synthesis, at birth, of several liver enzymes. This finding suggested that radiation may affect DNA transcription in mammalian tissue.

Since the induced synthesis of the rat liver enzyme tryptophan pyrrolase is dependent upon DNA transcription, we further investigated the effect of X irradiation on the induced synthesis of this enzyme in adult rats, in order to test the hypothesis that radiation can interfere with the transcription of the DNA template in resting mammalian cells. Radiation did interfere with the DNA-directed mRNA-dependent induction of this enzyme, but not with its in vivo stabilization by tryptophan. Although this finding suggested an impairment in the ability of irradiated liver cells to synthesize biologically effective, specific messenger RNA, it did not rule out the possibility of indirect, hormonally mediated, interference with protein synthesis by X irradiation. Such an effect, involving irradiation of the pituitary, was responsible for the interference observed by Heitbrink and DuBois with the synthesis of phosphorothioate oxidase in rat liver following X irradiation[3].

---

[1] Harrington, H. Effect of X-irradiation on the priming activity of DNA. Proc. Nat. Acad. Sci. U.S. 51:59-66 (1964).

[2] Pollard, E. C. Ionizing radiation: Effect on genetic transscription. Science 146:927-928 (1964).

The following experiment presents evidence that radiation interferes with the DNA-directed mRNA-dependent induction of tryptophan pyrrolase. The radiation effect was observed in whole-body, or body-only irradiated animals. Irradiation of the head only, with the body shielded, did not alter the normal induction process.

Intact, male, Sprague-Dawley rats weighing 120 grams (43 days old) were used in this study. Food and water were withheld from control and irradiated animals from the time of X irradiation to the time of sacrifice. Twenty-four hours after X irradiation (800 rad) experimental and control animals were injected with either hydrocortisone or tryptophan. Groups of animals were sacrificed at various time intervals up to 10 hours after injection of either hydrocortisone or tryptophan. Total enzyme activity was determined by our adaptation of the method of Knox[4]. The data, presented in the figures which follow, represent the mean of six replications of the complete induction curve. Standard errors are shown to provide an indication of the raw variability encountered in this study. The experimental design permitted analysis of variance with blocking by days to eliminate the effect of day to day variation.

Figure 2 presents induction curves following hydrocortisone administration obtained from control animals and animals receiving 800 rad, 250 kVP X-ray, total-body irradiation. The induced enzyme levels in the irradiated animals are lower than those observed in the controls. The difference between induction curves for control and irradiated animals is significant at the 1% level. Figure 3 presents induction curves following hydrocortisone administration obtained from control animals and animals receiving 800 rad X rays to the head only with the body shielded. Analysis of the data obtained revealed no significant difference between control and head-irradiated animals. Figure 4 presents induction curves following hydrocortisone administration obtained from control animals and animals receiving 800 rad to the body only, with the head shielded. Induced enzyme levels in the irradiated animals are lower than those observed in the controls. The difference between induction curves for control and irradiated animals is significant at the 1% level. Comparison of induction curves from whole-body and body-only irradiated animals receiving hydrocortisone revealed no significant difference. No differences were found among induction curves obtained from control, head-irradiated, body-irradiated, or whole-body irradiated animals injected with tryptophan. It thus appears that a radiation effect on the induction of tryptophan pyrrolase can be demonstrated only when induction is dependent on the postirradiation synthesis of new mRNA for this enzyme. Present results suggest that this effect is not indirectly mediated by hormonal factors but occurs within the cells of the liver. These findings are consistent with work suggesting that radiation interacts with DNA to produce alterations in the structure of this macromolecule. One may speculate further that this change in DNA structure may lead to the production of less biologically effective mRNA which in turn might result in the synthesis of inactive protein molecules.

---

[3] Heitbrink, B. E. and DuBois, K. P. Influence of partial-body X-irradiation on development of phosphorothioate oxidase in the livers of male rats. Radiation Res. 27:669-675 (1966).

[4] Mishkin, E. P. and Shore, M. L. Inhibition by actinomycin D of the induction of tryptophan pyrrolase by hydrocortisone. Biochim. Biophys. Acta 138:169-174 (1967).

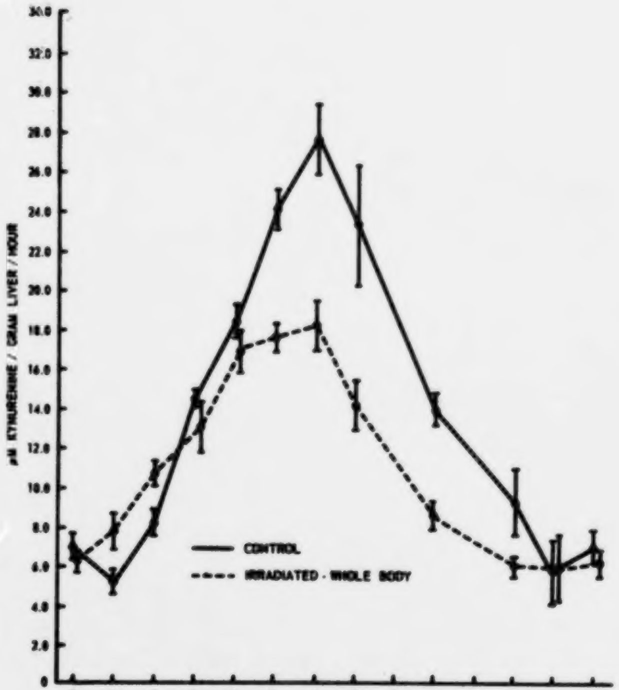


Figure 2. Induction Curves Following Hydrocortisone Administration and Whole-Body Irradiation.

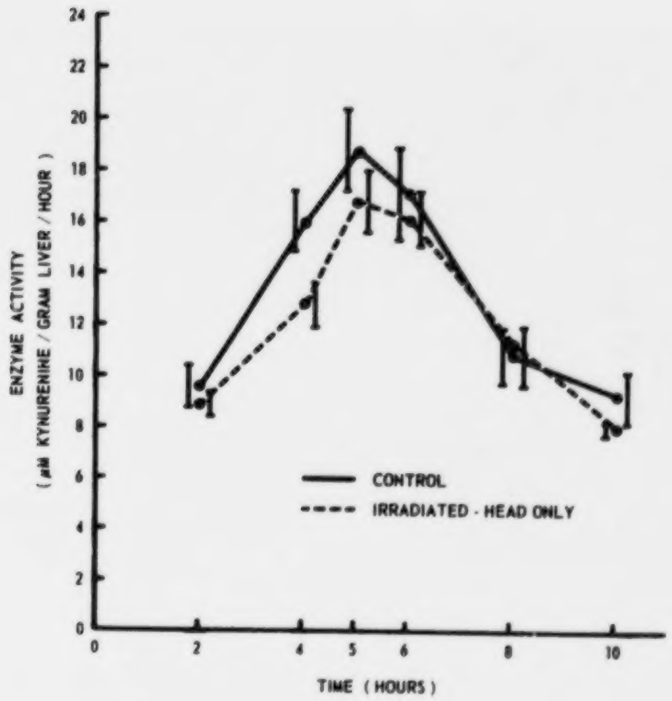


Figure 3. Induction Curves Following Hydrocortisone Administration and Head Only Irradiation.

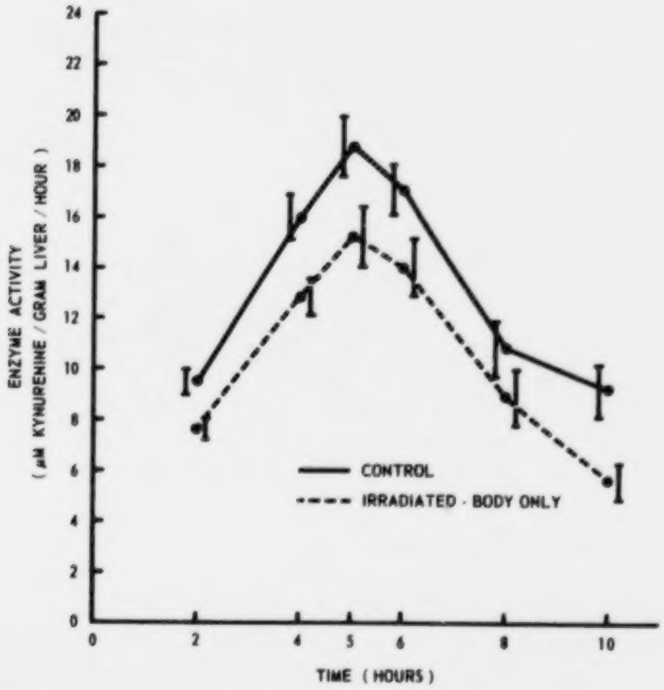


Figure 4. Induction Curves Following Hydrocortisone Administration and Body Only (Head Shielded) Irradiation.

B. RELATIVE RATES OF TRYPTOPHAN PYRROLASE DEGRADATION FOLLOWING THE ADMINISTRATION OF HYDROCORTISONE AND TRYPTOPHAN TO INTACT RATS

M. L. Shore, E. P. Sheppard

Tryptophan pyrrolase has been of considerable interest because it was one of the first mammalian enzymes found to be adaptive. The increase in the level of this enzyme which is observed after the administration of either the hormone, hydrocortisone, or the enzyme substrate, tryptophan, is inhibited by puromycin, thus implicating enzyme synthesis *de novo* in this process. Since increase in activity of this enzyme following hydrocortisone administration is prevented by simultaneous injection of actinomycin D, an antibiotic that interferes with messenger RNA (mRNA) synthesis, it has been assumed that synthesis of new mRNA is required for hormonal induction.[5] Also, the increase in tryptophan pyrrolase following administration of substrate is unaffected by the simultaneous injection of actinomycin D.[5] Thus, it is presumed that enzyme synthesis *de novo* occurs when either of these inducers is administered, but only in hormonal induction is new mRNA synthesis involved.

Schimke *et al.*[6] found that tryptophan, given after enzyme levels had been markedly elevated by multiple injections of hormone and substrate, prevents the subsequent normal decrease in activity of the enzyme. They therefore suggested that the substrate may act to stabilize the enzyme.

The present study, utilizing intact rats, presents data concerning the effect of hydrocortisone and tryptophan administration on the rate of degradation of tryptophan pyrrolase. We observed that the degradation of tryptophan pyrrolase increased markedly after the administration of hydrocortisone. Following tryptophan administration the normally observed increase in enzyme degradation was inhibited.

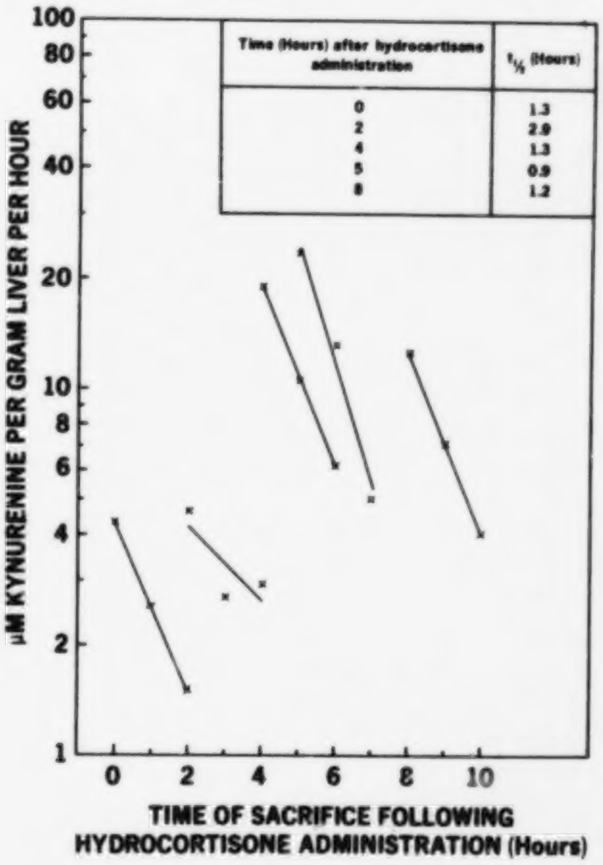
The male, Sprague-Dawley rats of 150 grams used in this study were allowed food and water *ad libitum* prior to experimental treatment. At various times following administration of either hydrocortisone or tryptophan, puromycin was injected intraperitoneally. Animals were sacrificed at 0, 1, and 2 hours after the initial puromycin injection. Tryptophan pyrrolase levels were determined by our adaptation of the method of Knox.[4]

Figure 5 presents results obtained when puromycin was administered to rats at various times following induction of tryptophan pyrrolase by hydrocortisone. Since puromycin prevents protein synthesis, the decrease in enzymatic activity observed after puromycin administration provides a measure of the relative rate of enzyme degradation. Satisfactory linear fits were obtained by the method of least squares in all cases with the exception of data obtained 2 hours after hydrocortisone administration. Half times ($t_{1/2}$) and the values of the slopes (k_e) were obtained for each of the lines; the value $k_e = 0.693/t_{1/2}$ can be used as a relative measure of the rate of degradation of the enzyme and provides an estimate of the rate constant for degradation.

---

[5] Greengard, O., Smith, M. A., and Acs, G. Relation of cortisone and synthesis of ribonucleic acids to induced and developmental enzyme formation. J. Biol. Chem. 238:1548-1551 (1963).

[6] Schimke, R. T., Sweeney, E. W., and Berlin, C. M. The analysis of kinetics of the rat liver tryptophan pyrrolase induction: Significance of both enzyme synthesis and degradation. Biochem. Biophys. Res. Commun. 15:214-219 (1964).

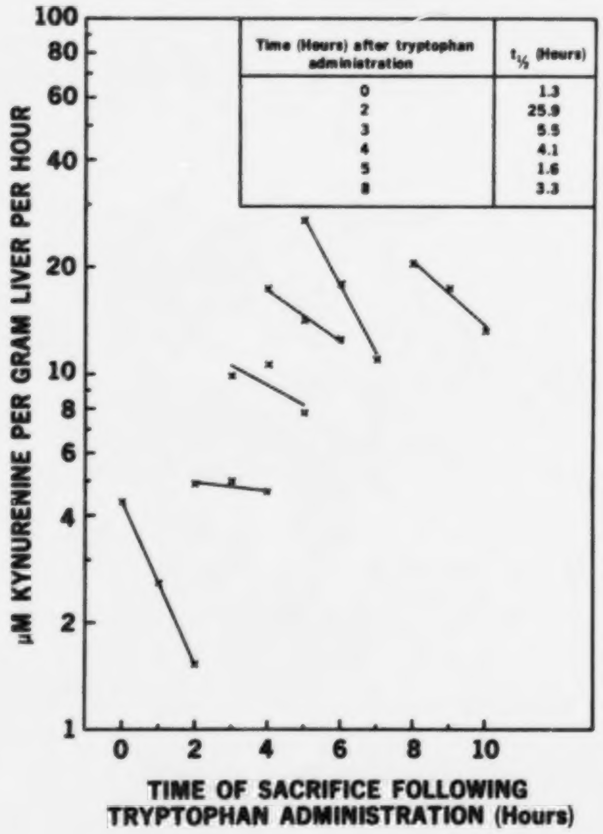


Time (Hours) after hydrocortisone administration	$t_{1/2}$ (Hours)
0	1.3
2	2.9
4	1.3
5	0.9
8	1.2

Figure 5. Results of Administering Puromycin to Rats Following Tryptophan Pyrrolase Induction by Hydrocortisone.

Hormonal induction of tryptophan pyrrolase results from an imbalance between synthesis and degradation, in which the rate of synthesis exceeds the rate of degradation. Since the half-time for degradation appears to remain relatively constant throughout, and enzyme levels increase markedly during enzyme induction, it is clear that hormonal induction must involve an increase in the absolute amount of enzyme being synthesized per unit time.

Quite a different picture is obtained when the disappearance of enzyme activity is examined after puromycin administration at various times following "induction" of the enzyme by its substrate tryptophan (Figure 6). The data were treated in the same way as the hydrocortisone data. In rats that did not receive tryptophan (0 time), the half-time of enzyme disappearance was 1.33 hours. Two hours after the administration of tryptophan, the half-time was 25.9 hours, indicating that degradation was considerably diminished. The half-times following tryptophan administration were compared with those observed following hydrocortisone injection. In every case (2, 4, 5, and 8 hours after the administration of the inducer), the half-times for enzyme degradation were significantly longer for animals receiving tryptophan. The values of k_e for degradation following hydrocortisone or tryptophan administration are given in Table 1. In every case where comparisons can be made the estimated rate constant for degradation was significantly decreased during tryptophan induction relative to hydrocortisone induction.



Time (Hours) after tryptophan administration	$t_{1/2}$ (Hours)
0	1.3
2	25.9
3	5.5
4	4.1
5	1.6
8	3.3

Figure 6. Disappearance of Enzyme Activity After Puromycin Administration.

TABLE 1. RATE CONSTANTS (k_e) FOR DEGRADATION OF TRYPTOPHAN PYRROLASE DURING HORMONAL AND SUBSTRATE INDUCTION OF THE ENZYME

Hour[a]	Hydrocortisone (k_e ± S.E.)	Tryptophan (k_e ± S.E.)
0	-0.522 ± 0.051	-0.522 ± 0.051
2	-0.239 ± 0.053	-0.027 ± 0.091[b]
3	-	-0.127 ± 0.096[b]
4	-0.547 ± 0.073	-0.171 ± 0.047[c]
5	-0.762 ± 0.062	-0.441 ± 0.091[c]
8	-0.564 ± 0.090	-0.209 ± 0.082[c]

a. Time after administration of inducer at which k_e was determined.
b. Value for k_e not significantly different from 0.
c. Value of P for differences between k_e's for hydrocortisone and tryptophan induction <0.01.

Thus it appears that hydrocortisone acts in the induction of tryptophan pyrrolase by increasing the rate of synthesis of the enzyme with no appreciable change in the rate of degradation, while tryptophan acts to decrease the rate of degradation, an effect which, following tryptophan administration, would lead to increased levels of the enzyme. The expected levels, which would be attained if synthesis of tryptophan pyrrolase were continued at basal rates, would not be as high as those actually observed 5 hours after tryptophan administration. Thus, these data suggest that tryptophan induction involves both a decrease in enzyme degradation as well as an increase in enzyme synthesis.

C. RADIATION EFFECTS ON PROTEIN SYNTHESIS IN THE CELL-FREE SYSTEM

W. A. Mills, L. R. Simmons

Our investigation of protein synthesis in the cell-free system, isolated from rat liver, has involved studies using both in vitro and in vivo irradiation.

The in vitro studies were focused on protein synthesis as a function of radiation dose delivered to the cell-free system which was characterized as to the optimum concentration of ribosomes and cell sap. Two doses were given, 17.5 krad and 35.0 krad to a system capable of in vitro protein synthesis. No differences in protein synthesis were observed.

Studies involving irradiation of the whole animal and subsequent isolation of the components capable of protein synthesis in vitro are also being studied. Animals were irradiated at doses of 400 rad, 600 rad and 800 rad; the ribosomes and cell sap were isolated at intervals as long as 8 weeks after exposure. Preliminary results are shown in Figure 7. To eliminate some of the uncertainties in these data, a more complete experimental design has been formulated, including a determination of the $LD_{50/30}$ for X-irradiated Sprague-Dawley rats maintained under our laboratory conditions.

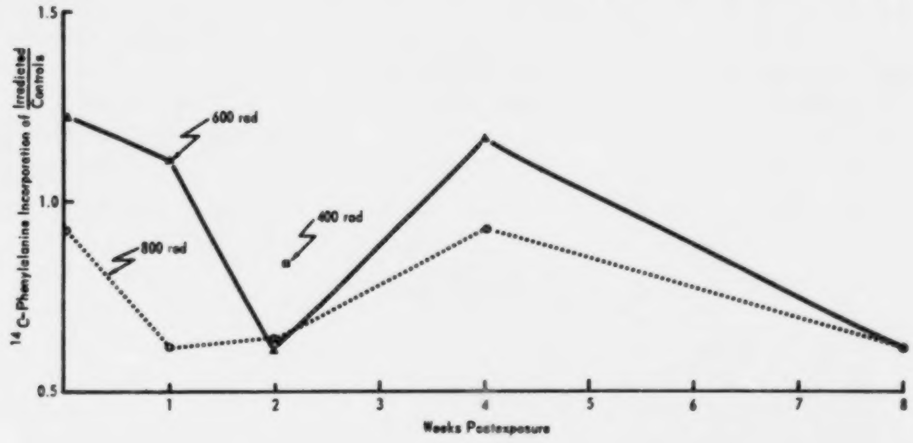


Figure 7. ^{14}C-Phenylalanine Incorporation by Irradiated Rats.

D. BIOCHEMICAL EFFECTS IN RATS IRRADIATED *IN UTERO*

D. G. Hazzard, T. J. Withrow

Ionizing radiation is known to cause profound effects on the developing embryo. Most *in utero* irradiation studies have been of a morphological nature dealing with gross abnormalities or death. It is probable, however, that at certain stages of development and at lower exposures, biochemical changes will occur that do not immediately manifest themselves as morphological changes. It is through alterations such as these that insight into the mechanism of radiation damage and the assessment of radiation risk are sought. Enzymes are proteins of a specific nature that are easily assayed and which could possibly provide sensitive measures of radiation effects.

It has previously been shown in this laboratory[7] that rats irradiated *in utero* with 180 rads on day 15 of gestation did not show the marked increase in activity of certain enzymes normally seen at birth. Other work in this laboratory has demonstrated that induced tryptophan pyrrolase synthesis in the rat is depressed following irradiation when such induction requires synthesis of new DNA directed mRNA subsequent to the irradiation.

We have begun experiments to determine whether the *de novo* enzyme synthesis at birth is dependent on new synthesis of DNA directed mRNA. These studies will provide an insight into the possible locus of the radiation effect, and may either implicate or eliminate radiation-induced alterations in DNA as a factor in the observed depression in enzyme synthesis at birth following irradiation *in utero*.

In another part of the project rats are irradiated at different times to find the period of gestation most sensitive to irradiation damage as measured by changes in the *de novo* synthesis of certain enzymes in the liver at birth. The enzymes being utilized in this study are lactic dehydrogenase, glycerophosphate dehydrogenase, and glucose-6-phosphatase.

---

[7] Smith, C. H., Shore, M. L. Impaired development of rat liver enzyme activities at birth after irradiation *in utero*. Radiation Research 29:499-504 (1966).

E. RADIATION EFFECTS ON THE STRUCTURE OF GLOBULAR PROTEINS IN SOLUTION

D. E. Janes, Jr.

The biological activity of protein is directly related to protein structure; changes in structure lead to changes in activity. Radiation acts on structure in two ways. First, it can alter the structure of synthesized protein as demonstrated by the in vitro radiation-inactivation of enzymes. Second, it can cause mutations in DNA that lead to changes in the amino acid sequence of subsequently synthesized protein. About 100 so-called "base substitution" mutant proteins are known, i.e., proteins which differ from the native form by a single amino acid and can be accounted for by the alteration of a single base in the relevant nucleic acid. Little information is available on the solution conformation of the mutant proteins, or the comparison under stress of their conformal changes with the conformal changes under stress of the native analogs.

The present study investigates the applicability of the technique of transient electric birefringence to determine small changes in the solution conformation of proteins and the correlation of conformational data with biological activity. Bovine pancreatic ribonuclease has been chosen as the model protein for these studies because the amino acid sequence and three-dimensional crystalline structure are known, the activity of the molecule is amenable to spectrophotometric assay, and the molecule has reduced but detectable activity after a number of treatments that are known to change its conformation. Currently work is under way to adapt the spectrophotometric assay of Crook et al.[8] for ribonuclease activity for use in this laboratory with mixed-solvent systems.

---

[8] Crook, E. M., Mathias, A. P., and Rabin, B. R. Spectrophotometric assay of bovine pancreatic ribonuclease by the use of cytidine 2':3'-phosphate, Biochem. J. 74:234-238 (1960).

F. FURTHER INVESTIGATIONS OF THE INFLUENCE OF GLUCAN ON IRRADIATED AND NONIRRADIATED MICE

R. K. Fred, J. G. Harris, T. M. Zaremba

Our experiences with administering the polysaccharide glucan to mice have indicated that it produces toxic side effects. It was commonly observed that 1 mg of glucan produced lethargy, twitching and rapid respiration beginning about 10 to 20 min. after its administration. Mice either recovered completely or died within the first hour. Mice irradiated with 5 - 10% lethal X or γ rays were found to be more vulnerable than nonirradiated mice to the glucan. In experiments with a total of 550 mice, glucan administration killed 6.3% of the nonirradiated mice and 16.5% of the irradiated mice; this difference is statistically significant ($P<0.001$). Our initial studies were aimed at determining the nature of the toxic reaction, but later studies also gave some insight into the mechanism of glucan stimulation of the reticuloendothelial system.

Glucan-treated mice appeared, on close examination, to be cyanotic compared to untreated mice. Furthermore, histological sections revealed that a substantial amount of the glucan was trapped in the interalveolar pulmonary capillaries. Both of these findings were commensurate with the gross symptoms of lethargy, twitching and rapid respiration. On this basis, it was reasoned that grinding the glucan into smaller particles would reduce the amount of glucan trapped in the lungs and possibly decrease its toxicity. Initial experiments indicated that grinding glucan for 3 to 4 days in a Pyrex glass mill partially reduced its toxicity. However, it was also found that grinding the glucan reduced its RE stimulatory activity.

The correlation between the toxic effects and the RE stimulating effects suggested the need to determine the differences in tissue distribution of ground and unground glucan. For this purpose 70 mg of biosynthetically ^{14}C-labeled glucan was extracted from Saccharomyces cerevisiae by the method used by Fleischmann Laboratories to prepare their glucan. Three glucan preparations were used in these experiments: Fleischmann's glucan, ^{14}C-labeled glucan, and a 50/50 combination of these two.

Figure 8 shows the tissue distribution of the ^{14}C-labeled glucan. This preparation was not ground and contained only glucan which had been produced in our laboratory. Figure 9 shows a similar pattern of tissue distribution for a nonground 50/50 mixture of Fleischmann's glucan and ^{14}C-labeled glucan. The distribution for nonground glucan indicated in Figure 9 and in Figure 8 is consistent with the tissue distribution for Zymosan which has been reported by Samoylina.[9] Figure 10 shows the tissue distribution for ground 50/50 labeled glucan.

---

[9] Samoylina, N. L. Distribution of ^{14}C-labeled Zymosan in different tissues after the administration, Probl. Gematol. Perel. Krovi 7:16 (1962).

Total recovery of the labeled glucan is low compared with the previous studies, although the percent activity in liver, spleen, and kidney is approximately the same as for nonground glucan. The lung definitely contains less labeled material. About 11% of the ^{14}C-label in the ground glucan preparation was associated with the supernatant after centrifugation at 32,800 g; however the fate of the unrecovered activity cannot be accounted for by this alone.

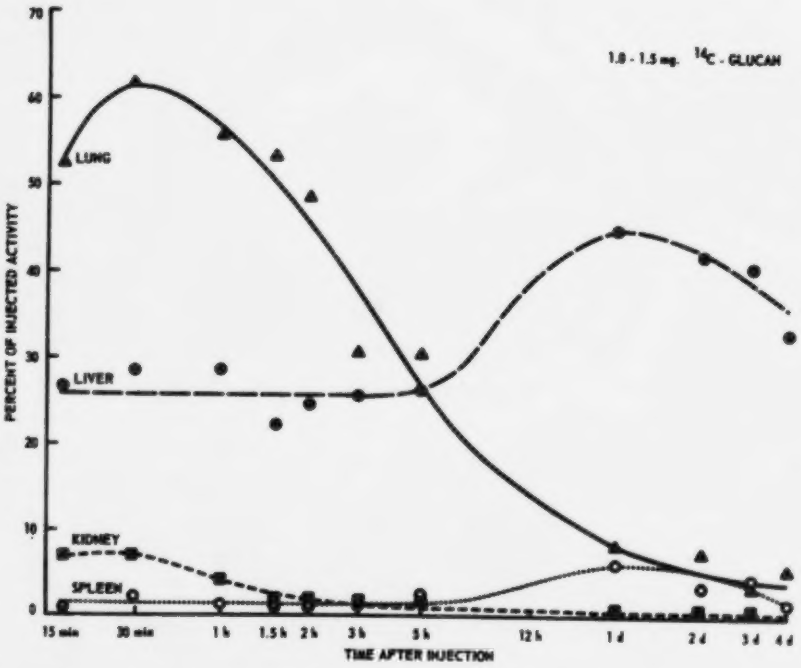


Figure 8. Tissue Distribution of ^{14}C-Labeled Glucan

The differences in physical size indicated by dispersion properties typical of the glucan preparations are shown in Figure 11. Grinding changes the microscopic appearance of glucan as indicated. The difference in the settling indicates that grinding decreases the effective mass of the glucan particles and therefore increases the surface area per unit mass. Table 2 indicates the results of the experiments to determine the RE stimulatory activity of the various glucan preparations. RE phagocytic capacity was determined 4 days after administering the glucan. These results confirmed our earlier observations that grinding reduced the RE stimulatory activity of glucan.

Preliminary work in our laboratory indicates that the symptoms of glucan toxicity can be prevented by placing the mice in an atmosphere of 95% oxygen and 5% carbon dioxide for an hour after glucan administration. Such oxygen treatment does not alter the RE stimulatory activity of the glucan.

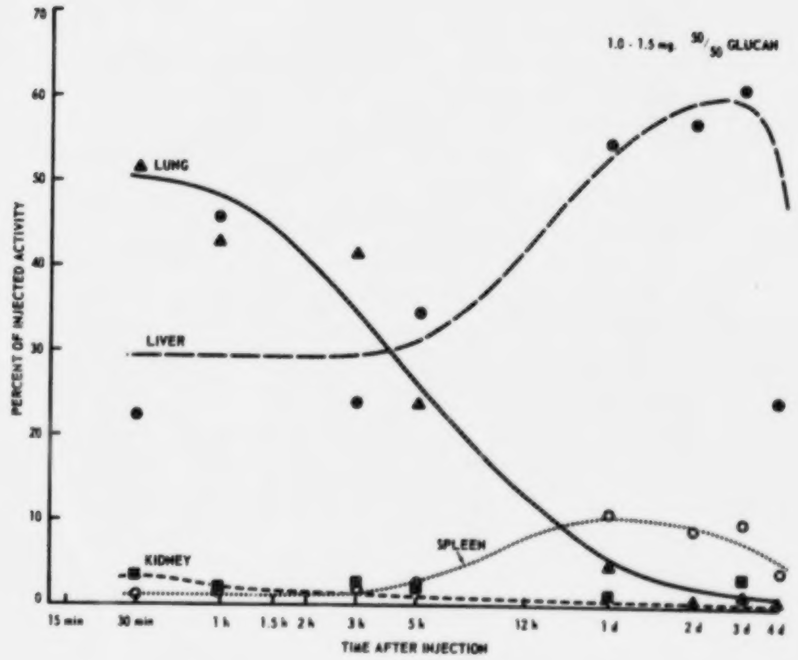


Figure 9. Tissue Distribution of Nonground Mixture of Commercial and ^{14}C-Labeled Glucan.

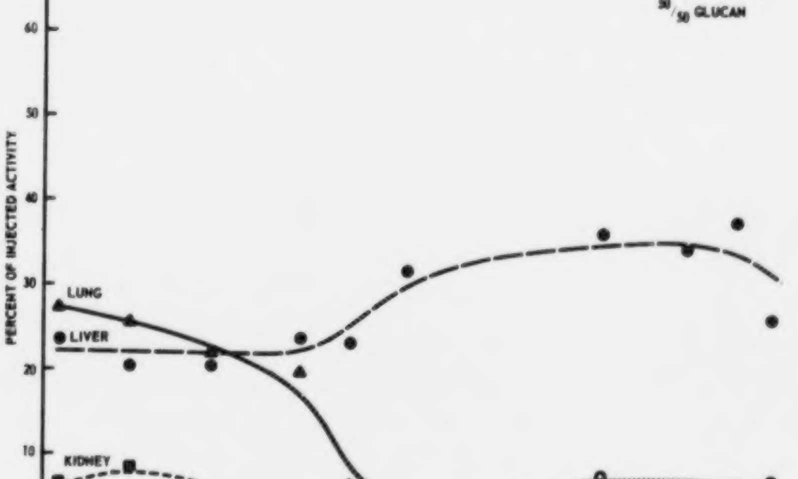


Figure 10. Tissue Distribution of Ground Mixture of Commercial and ^{14}C-Labeled Glucan.

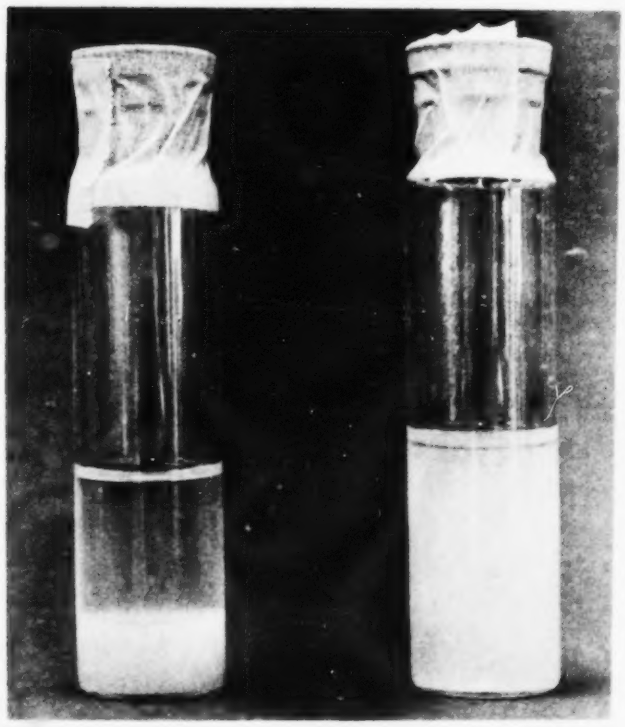


Figure 11. Differences in Physical Size Indicated by Dispersion Properties in Nonground (left) and Ground (right) Glucan.

TABLE 2. INITIAL RATE OF CARBON DISAPPEARANCE[a]

No. of Mice	Treatment	Amount of Glucan Injected, mg	Carbon Clearance, mg/min ± S.E.
34	Controls	-	0.1388 ± 0.0097
3	Ground Glucan	1.5	0.2816 ± 0.0273
5	50/50 Glucan	1.5	0.4590 ± 0.0635
11	50/50 Ground Glucan	1.5	0.3634 ± 0.0322
18	Glucan	1.0	0.4275 ± 0.0206
8	Ground Glucan	1.0	0.2847 ± 0.0271

a. All animals injected with 12 mg colloidal carbon.

These investigations support the hypothesis that respiratory involvement is an important factor in glucan toxicity. Irradiated mice are apparently more sensitive to the stress produced by glucan injection.

In these experiments we observed that the amount of glucan sequestered in liver is relatively independent of whether glucan has been ground or not. However, the stimulatory effect of glucan is significantly reduced by grinding.

It may therefore be possible that the particle size is a more important factor than chemical composition in determining its RE stimulatory activity. This is emphasized by the observation that increasing the glucan surface area by grinding causes a decrease rather than an increase in the RE activity, even though the amounts of glucan in the liver are similar for each of the preparations.

G. ISOLATION OF KUPFFER CELLS FROM RAT LIVER

R. K. Fred, T. M. Zaremba

Previous studies in this laboratory have indicated that sublethal whole-body irradiation of mice causes a decrease in the phagocytic capacity of the Kupffer cells.[10] To determine the site of radiation damage, it becomes important to investigate the effect of radiation on phagocytosis at the cellular level. This may be accomplished by isolating the Kupffer cells and determining the biochemistry and effects of radiation on in vitro phagocytosis.

Nearly 95% of the cells in the liver are parenchymal cells and Kupffer cells in a ratio of about 3:2.[11] Thus, if all of the parenchymal cells are removed, contamination of the suspensions by cells other than parenchymal would be minor. By using a modified combination of the methods of St. George,[12] and Garvey,[13] we have obtained Kupffer cell suspensions which are virtually free of parenchymal cells. The method consists of a liver perfusion, mincing, and incubation of the minced liver with trypsin. This method yields about 200-300 mg of Kupffer cells from each 10 grams of rat liver. Kupffer cells labeled in vivo with colloidal carbon have been examined, after isolation, with the electron microscope. A typical electron photomicrograph of a Kupffer cell is shown in Figure 12. The isolation method developed in this laboratory is a major improvement over previous methods, particularly because the yield of Kupffer cells is high and the procedure is rapid.

The oxygen uptake of the Kupffer cell suspensions separated from rat liver has been compared to that of whole liver cell suspensions. The oxygen uptake (Q_{O_2}) of these suspensions at various times is shown in Table 3. The Q_{O_2} of the Kupffer cell suspensions compares favorably with that of the whole liver suspensions. The oxygen uptake of both types of suspensions falls off rather quickly after 60 min. However, the oxygen uptake of Kupffer cells falls off more rapidly.

---

[10] Fred, R. K., and Shore, M. L. Application of a mathematical model to the study of RES phagocytosis in mice. In "Advances in Experimental Medicine and Biology", Vol. 1 "Reticuloendothelial System and Atherosclerosis" (N. R. DiLuzio and R. Paoletti, ed.) p. 1-17. Plenum Press, New York (1967).

[11] Daoust, R. The cell population of liver tissue and the cytological reference bases. In "Liver Function" (R. W. Brauer, ed.) p. 3-10. Waverly Press, Inc., Baltimore, Maryland (1958).

[12] St. George, S. Separation of reticuloendothelial and parenchymal cells of rat's liver by a new gentle technique. In "Reticuloendothelial Structure and Function" (J. M. Heller, ed.) p. 449-461. Ronald Press, New York (1960).

[13] Garvey, J. S. Separation and *in vitro* culture of cells from liver tissue. Nature 191(4792):972-974 (1961).

Figure 13 shows the oxygen uptake as a function of time for Kupffer cells, whole liver cells, liver homogenates, and heat-killed liver cells. The oxygen uptake of both homogenates and killed cells is much less than that of the intact cells. These results indicate that the Kupffer cells present in the suspension are viable.

Experiments with various types of media including Krebs-Ringer (phosphate), MEM (phosphate),[14] and L-15[14] have indicated that both L-15 and MEM which contain amino acids, vitamins, and salts, stimulate the oxygen uptake of Kupffer cell suspensions to a greater extent than does Krebs-Ringer which contains only salts. This suggests that Kupffer cells require amino acids and vitamins for maximum oxygen uptake. The in vitro system appears to have an optimum respiration rate at a pH of 7.6-7.8.

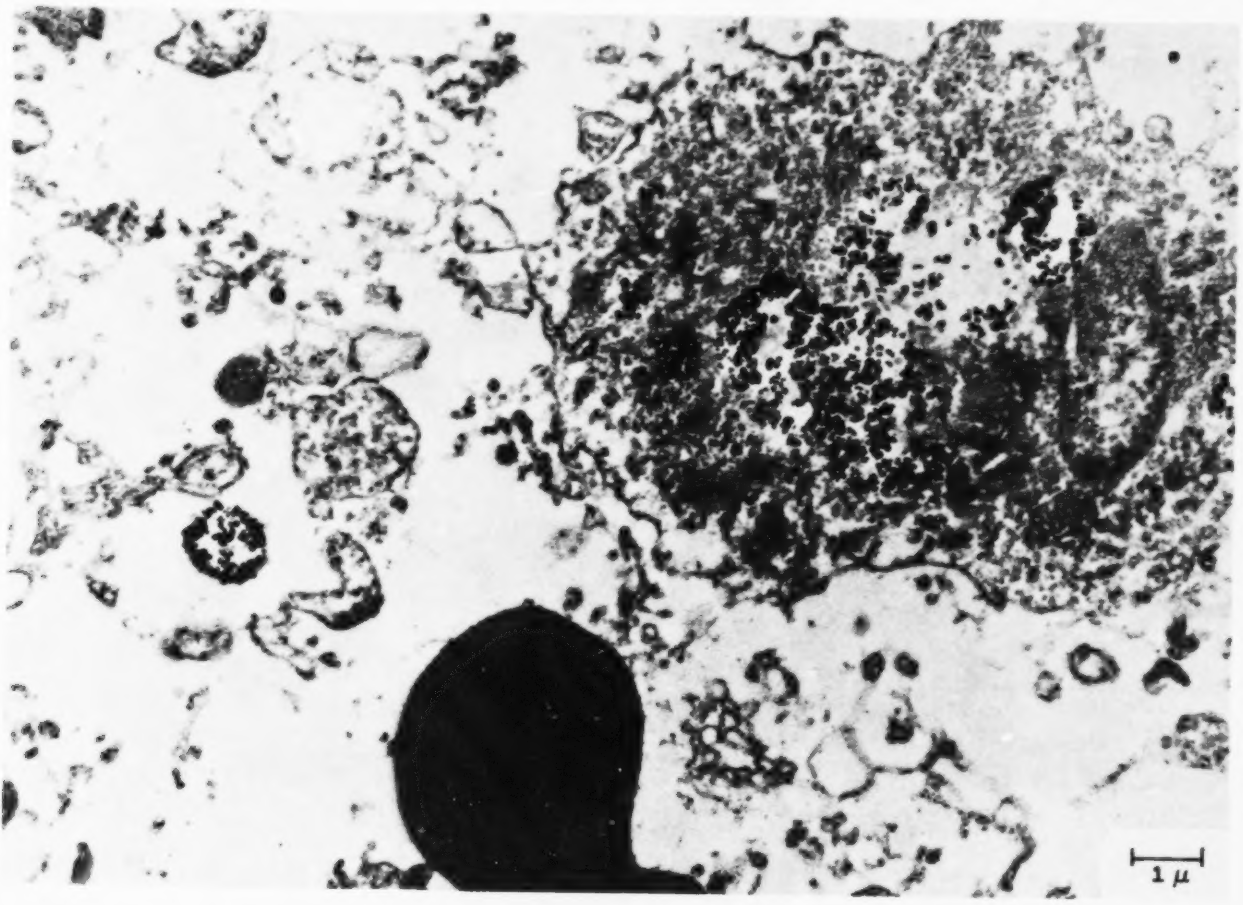


Figure 12. Electron Photomicrograph of an Unstained Kupffer Cell Containing Carbon Particles.

---

[14] Obtained from Flow Laboratories, Rockville, Maryland.

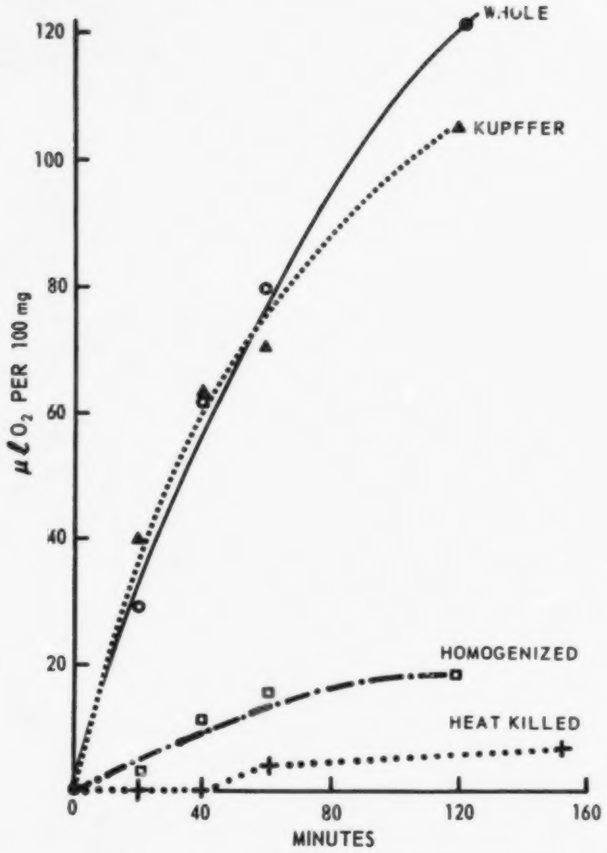


Figure 13. Oxygen Uptake of Various Liver Cell Suspensions

TABLE 3. MICROLITERS OF OXYGEN (Q_{O_2}) TAKEN UP PER MILLIGRAM OF DRY TISSUE PER HOUR

Incubation Time, min	Whole Liver Cells	Kupffer Cells
10	29.9	29.9
60	21.6	14.6
120	11.0	6.5

II. RADIONUCLIDE TOXICOLOGY LABORATORY

J. F. Stara, Chief

During the past year the Radionuclide Toxicology Laboratory conducted studies that lie in three main areas of interest. The bio-effects studies are concerned primarily with strontium-85, -89, and -90 metabolism in felines, and the effects of radiostrontium in the developing felines. These studies were initiated to obtain needed radiostrontium kinetics and dosimetry data in felines prior to a long-term effect study on strontium-induced leukemia. This latter study was initiated in the second half of this fiscal year. Also of prime concern are iodine-131 dosimetric and teratogenic studies in guinea pig embryos.

The second area of interest is the radiopharmaceutical metabolism studies designed to contribute to the field of nuclear medicine both from the standpoint of improving diagnoses and (in case of radiostrontium) of reducing body burden by dietary supplements. These studies have been discontinued due to a lack of financial support. Phases that are nearing completion are reported herewith.

The third area, metabolism and assessment of heavy radionuclides, includes analyses of uranium miners and Alaskan Eskimo tissues for polonium 210 and lead 210, and initial polonium-210 translocation studies in guinea pigs and cats. These studies have been concerned with determining radiation hazards in selected populations, such as uranium miners. Three animal support studies were initiated to clarify basic questions on polonium-210 and lead-210 tissue kinetics.

A. BIO-EFFECTS STUDIES

1. Radiostrontium Metabolism and Effects in Cats During Development and Growth

J. F. Stara, N. S. Nelson, R. G. Wolfangel, L. S. Rosenstein

a. Placental Transfer of Divalent Cations

N. S. Nelson

Pregnant dams must be restrained during surgical procedures by a means of narcosis that will not significantly alter the metabolic system, especially rates of placental cation transfer. One method of restraint we have investigated employs pin electrodes in the temporal region and results in various states of electronarcosis. Although this method has proved satisfactory when the animal was pre-tranquilized, without tranquilization it was not entirely successful.

The major benefit of using electronarcosis is its minimal effect on normal physiology while holding an animal in a narcotized state for several hours. We are therefore investigating other types of electrodes (pad or plate), and other locations of electrode placement (frontal-occipital, frontal-vomeral regions) to obtain consistently reproducible and controllable electronarcosis.

b. Strontium-85, -89, and -90 Kinetics in Cats

J. F. Stara, R. G. Wolfangel, L. S. Rosenstein

Experimental kinetics of strontium 85 and 90 in 12 age groups of felines have been studied for the past 3 years. Each age group has been subdivided according to the method of isotope administration--single dose, chronic uptake, and retention pattern following chronic labeling from conception. Using whole-body counting methods, the strontium-85 body burdens have been regularly assessed for all the age groups regardless of the method of strontium-85 administration. Daily observations from almost 400 animals are nearly complete and statistical analyses using several computer subprograms have begun. Similar data are being gathered for cats in embryonic stages of development. The results are expected to offer a thorough and exhaustive picture of strontium metabolism in the feline.

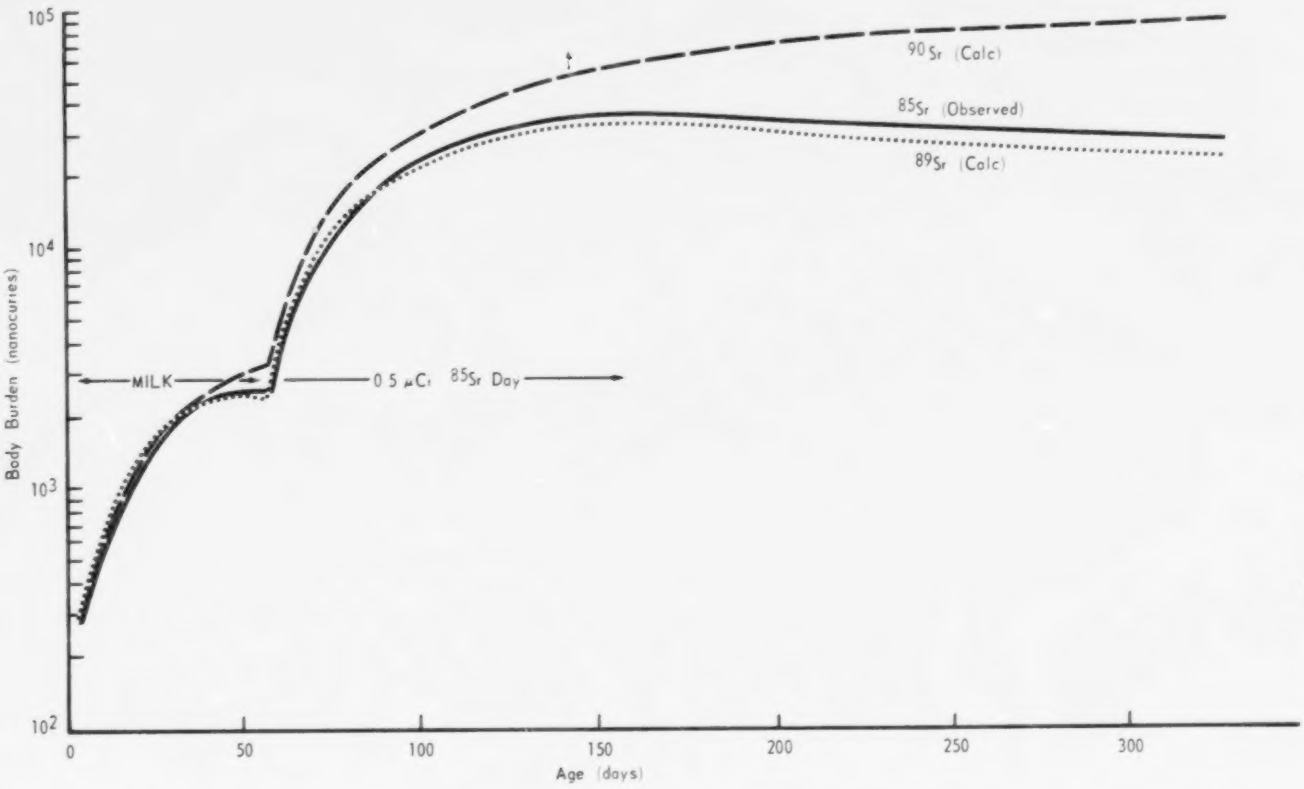


Figure 14. Strontium-89 and -90 Uptake Curves Calculated from Strontium-85 Data.

A new study is concerned with investigating uptake and retention of strontium 89. Analysis of strontium-85 data with appropriate corrections for differences of physical decay can be used to describe strontium-89 or strontium-90 uptake and retention. Extrapolating uptake data obtained from cats dosed daily with strontium 85 to a corresponding predicted strontium-89, -90 uptake curve is complicated by the biological treatment of the radioisotope within the organism (Figure 14).

However, we have made adjustments to the curve by considering the cumulative uptake curve to be the superposition of the regular dietary intake of the nuclide on the biological and inherent physical decay of the nuclide. With the aid of a high-speed digital computer (IBM 7040) to perform the required calculations, we were able to plot the curves in Figure 14 based upon observed strontium-85 data.

c. Influence of Diet Composition on Skeletal Deposition of Strontium

N. S. Nelson, R. G. Wolfangel, J. F. Stara

During the past 4 years, three different diets containing strontium 85 have been fed to respective groups of developing felines. Each of the test diets differed significantly in its content of stable strontium, calcium, and phosphorus. At sacrifice, tissues obtained from the test cats were analyzed for calcium, strontium, and inorganic phosphorus (Pi); in addition, the specific activity of strontium was determined (μCi ^{85}Sr/mg Sr). The mineral values obtained for the cat bones have been compared to the mineral composition of their respective diets (Table 4).

The strontium concentration in bone ash was decreased by increasing the calcium content of the diet, whereas differing stable strontium levels in the diet failed to produce any recognizable variation of bone strontium concentrations in this experiment. Calcium and phosphorus retention in the feline bones was nearly identical for each dietary group, indicating that each of the diets produced an ample level of minerals to maintain the health of the animals. Observed ratios (O.R. bone/diet) were similarly dependent on the relative concentration of strontium and calcium in the felines' diet. Comparisons of radiostrontium specific activities between bones and the diet are in progress.

Strontium-85 uptake for two dietary groups is illustrated in Figure 15. Each feline received 0.5 μCi of radiostrontium per day along with the test diet. The body burden of radiostrontium in the Purina dietary group (2.126% Ca) was nearly one-half of the body burden observed for the cats fed the C/D diet (0.236% Ca) at the end of a 64-day period of radiostrontium feeding.

TABLE 4. Ca^{++}, Sr^{++}, AND Pi[a] IN DIET AND BONE OF FELINES

Type of Diet	Minerals in Diet, %			Age at Sacrifice, days	Minerals in Ash of Long Bones, %			O.R. Bone/Diet
	Sr^{++}	Ca^{++}	Pi		Sr^{++}	Ca^{++}	Pi	
C/D FY 63-67	0.000149	0.160	0.160	156 to 175	0.0292 ±0.0010	37.60 ± 0.20	17.60 ± 0.20	0.839
C/D FY 68	0.000440	0.236	0.191	151 to 220	0.0259 ±0.0003	34.69 ± 0.47	17.59 ± 0.35	0.401
Purina Cat Chow	0.002290	2.126	1.303	210 to 220	0.0217 ± 0017	35.39 ± 0.71	17.76 ± 0.98	0.565

[a] Inorganic phosphorus.

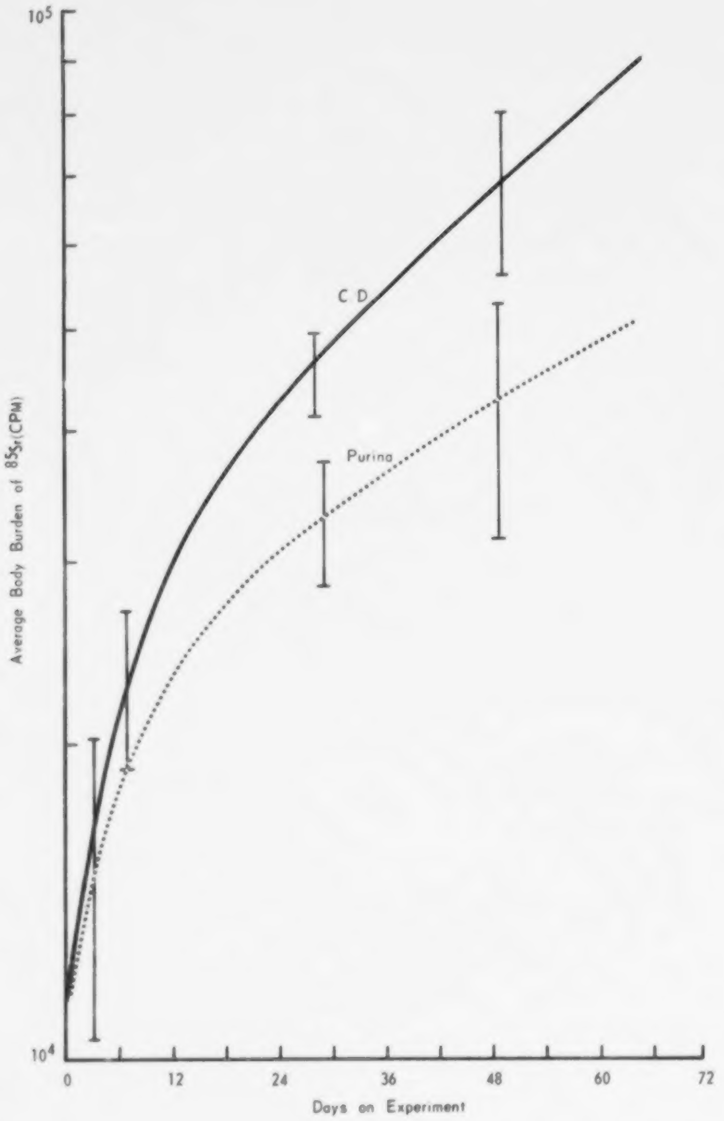


Figure 15. Strontium-85 Uptake in Felines on Two Diets.

The influence of dietary mineral composition on strontium retention illustrated in Figure 15 indicates that the strontium body burden can be controlled within limits by selecting a diet with a prescribed mineral content. Also, these data show that for any long-term experiments involving kinetics of radiostrontium, the mineral content of the diet must be uniform to minimize variations in the results.

d. Strontium-90 Dosimetry in Skeletal Tissues of Cats

L. S. Rosenstein, N. S. Nelson, J. F. Stara

The strontium-90 dosimetry studies this year have concentrated on refining the proper autoradiographic materials and instrumentation for a very precise evaluation of bone dose parameters. Consequently, several nuclear emulsions were calibrated and some of their more important characteristics were listed. Upon completion of the emulsion study, Kodak NTB series track plates of 10μ thickness were selected to be used in the skeletal studies. The emulsion itself showed excellent sensitivity and latent image stability, fair resolution, and a negligible amount of fade (4%). It also provided us with a linear response over a relatively wide density band. Using this emulsion, some thick section bone autoradiographs were made. The resulting dose rate patterns were evaluated using a Canalco Microdensitometer and a Joyce Loeble Isodensitracer.

The preliminary results obtained using the Canalco instrument intimated that a minimum of 7,000-10,000 data points would be necessary to provide an IBM 1130 digital computer/incremental plotter with enough information to predict the dose rate patterns. However, the Canalco instrument is not fully automated and lacks proper facilities for easy specimen-computer plot referencing. The Joyce Loeble instrument on the other hand is particularly versatile in that it allows one either to plot the contours directly or to select individual sections and plot density profiles. An example of the output from this device is shown in Figure 16. The dose contours at the right of the autoradiogram have been drawn directly from the isodensitracer surface patterns.

e. Investigation of Strontium in Tooth Layers as an Index of Strontium Skeletal Burdens

J. F. Stara, J. Dvorak, J. M. Cooper

Efforts to assess strontium skeletal burdens by gross analyses of extracted teeth for strontium content have been reported, but the results have been variable. This is understandable since the mineral turnover in respective teeth fractions varies from extremely slow in enamel, to relatively rapid in forming cementum. We have begun an experiment to compare the densities of the various teeth fractions to densities of the various bone types in an attempt to define a relationship between teeth and bone tissues.

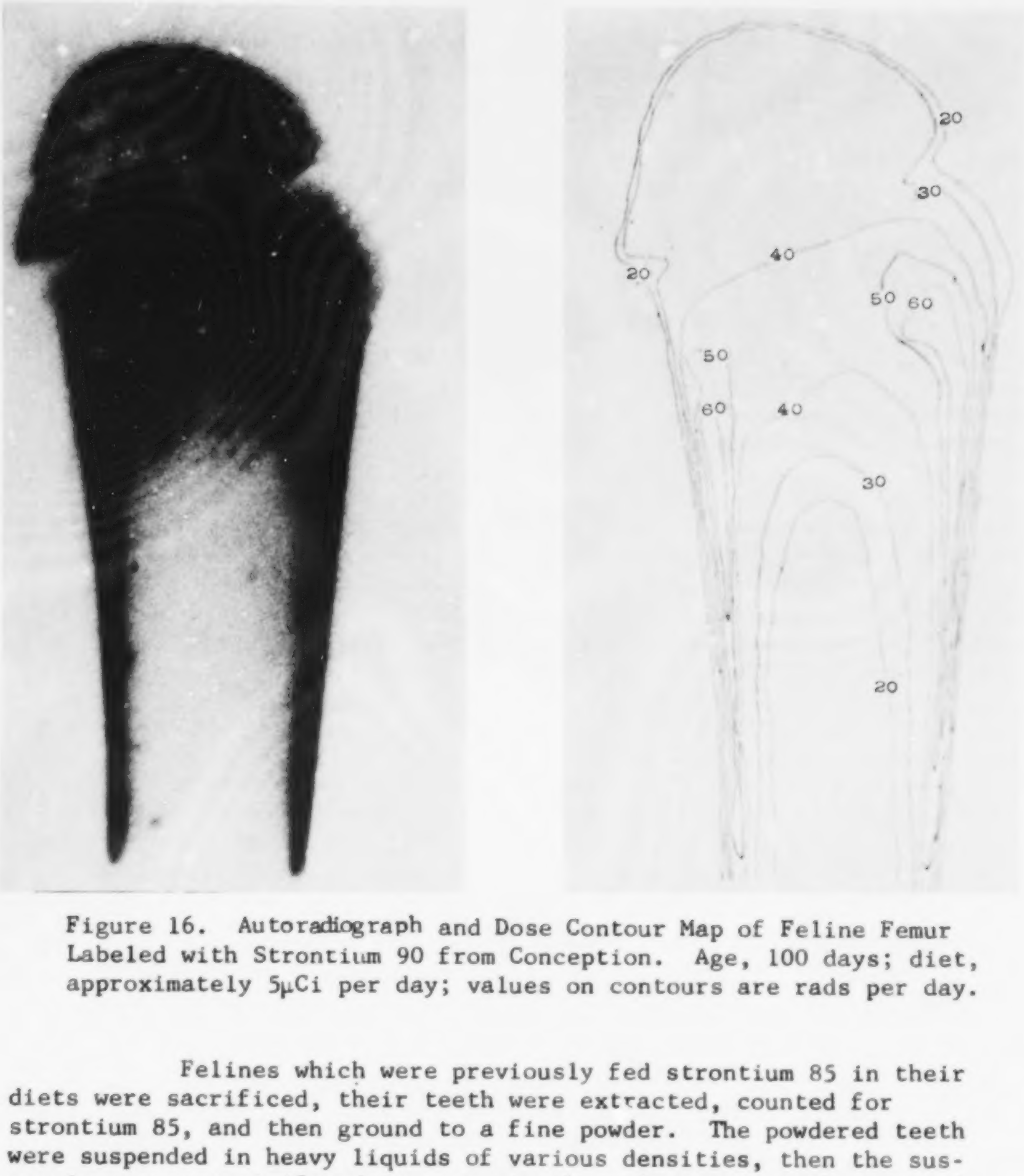


Figure 16. Autoradiograph and Dose Contour Map of Feline Femur Labeled with Strontium 90 from Conception. Age, 100 days; diet, approximately 5μCi per day; values on contours are rads per day.

Felines which were previously fed strontium 85 in their diets were sacrificed, their teeth were extracted, counted for strontium 85, and then ground to a fine powder. The powdered teeth were suspended in heavy liquids of various densities, then the suspensions were centrifuged to separate the pulverized teeth into different density fractions. The variable density liquids were prepared by mixing tetrabromoethane and acetone in various proportions (i.e., 56.11 to 43.89 for a density of 2.01, and 78.1 to 21.9 for a density of 2.49). The teeth were separated into distinct density fractions except for the density range between 2.05 and 2.20, which represents the two types of dentine and possibly a small percent of highly mineralized cementum (Figure 17). The enamel layer of deciduous teeth in very young animals varied in density; however, the enamel layer of permanent teeth was consistently separated at a liquid density of 2.5. Cementum was routinely separated using a liquid density varying from 2.01 to 2.02. The remaining tooth fraction was obtained between a density of 2.05 and 2.20, but within this density range the layer appeared to be a composite of two distinct subfractions: Dentine I, with a density range of 2.10 to 2.20, and Dentine II with a density range of 2.05 to 2.10. Dentine II probably contained a small portion of highly mineralized cementum; i.e., particles from the junction of both layers. Cortical bone samples obtained from the same animals as the teeth, were subjected to density determinations similar to the teeth fractions. These values fell in the Dentine II range.

The analyses of teeth and bone fractions for strontium 85 specific activity (μCi ^{85}Sr/mg Sr++) and strontium to calcium ratio are in progress. Completion of these analyses is necessary before determining if levels in tooth fractions can be used as a reliable index of strontium skeletal burdens.

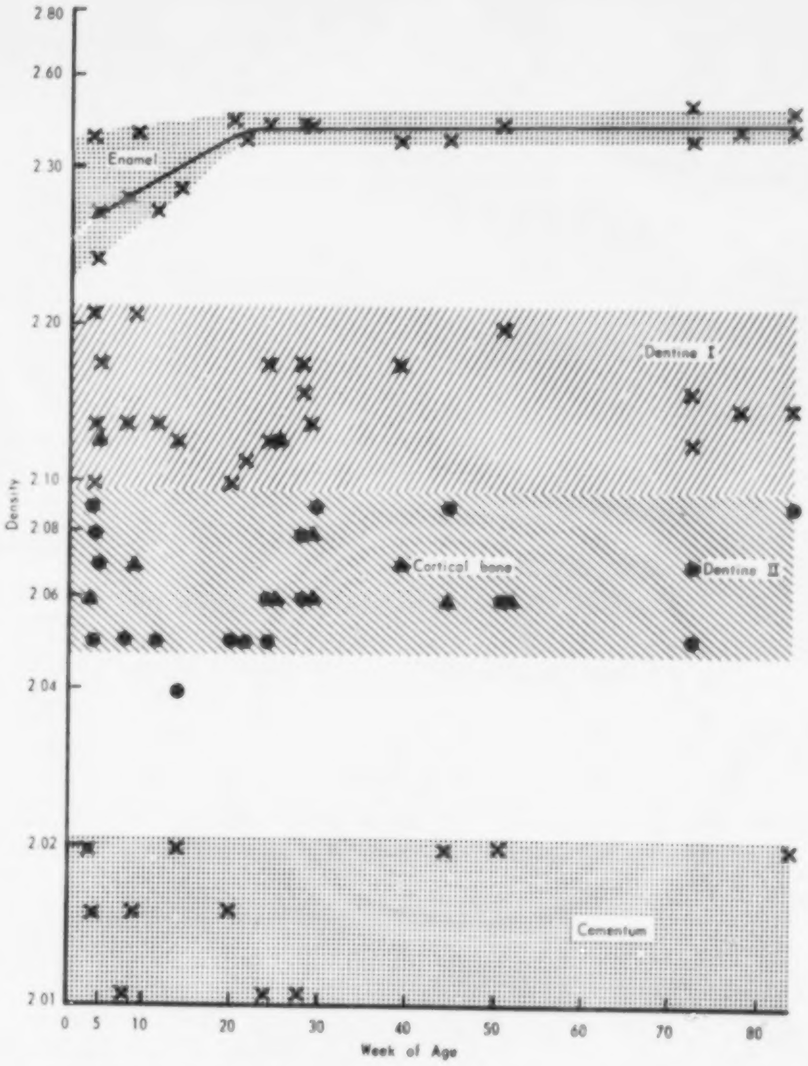


Figure 17. Densitometric Separation of Tooth Fractions from Felines.

2. Radioiodine Distribution in Guinea Pigs and Felines

a. Maternal-Fetal Relationship of Iodine 131 in the Guinea Pig

J. F. Stara, N. S. Nelson, R. M. Hoar, A. H. Wolff

Continued investigation of iodine-131 metabolism in guinea pigs has allowed some refinement of earlier data. Because the retention of iodine 131 in adult female guinea pigs is similar in both pregnant and nonpregnant females, the values for both groups of animals can be pooled. Both the biological and effective "whole body" retention in guinea pigs reflected a two-component exponential system (Figure 18). Biological half-life ($T_{1/2}$) of radioiodine in the thyroid as observed by probe counts of the thyroid area of the neck show a single component exponential, the half-life of which is slightly shorter than the biological $T_{1/2}$ of the long "whole body" component. Final values for biological iodine-131 retention in the guinea pig at 24 hours averaged 15.5% with an initial rapid component ($T_{1/2}$ = 4.14 days) and a second slower component ($T_{1/2}$ = 26.25 days); biological retention in the adult thyroid had a $T_{1/2}$ of 22.7 days.

The excretion pattern changed from an initial urine to feces (U/F) ratio of approximately 40:1 at 24 hours, to a constant 3:1 ratio about one week after the animals were given a single dose of iodine 131.

The rate of weight and iodine-131 activity increase in the fetus is shown in Figure 19. The values for gestation days 10-14 are for the egg cylinders because we could not separate the egg cylinder into individual tissues during this time. After 14 days of gestation, it is possible to separate the egg cylinder into embryos, placenta and decidua, so that total activity for the embryo is shown. The curve of fetal weight increase is parallel to published data. The decrease in iodine-131 activity shown by the egg cylinder at 13 days may be a reflection of the lag in vascularization as the embryo shifts from a yolk sac placenta to an allantoic placenta. Data reflecting the relationship of maternal plasma and maternal thyroid to embryonic tissues were collected and analyzed. In general, the results indicate that radioiodine was concentrated on the fetal side as shown by iodine-131 concentration in the placenta vs. maternal decidua. On a per gram basis, the iodine-131 concentration in the fetal placenta was ten times greater than that of the maternal plasma. The increase in iodine-131 activity in the embryo and corresponding change in slope between days 20 and 22 reflect the onset of thyroid function. This was supported by histological investigations of the developing thyroid employing special follicular stains. Complete studies involving separation of iodine-131 activity in the embryonic tissues into various organic compounds using chromatographic methods are in progress. Other complementary studies including microdensitometric evaluation of radiation dose as determined from the autoradiographs of sections and evaluation of thyroid area of the embryo by radioassay have not been completed.

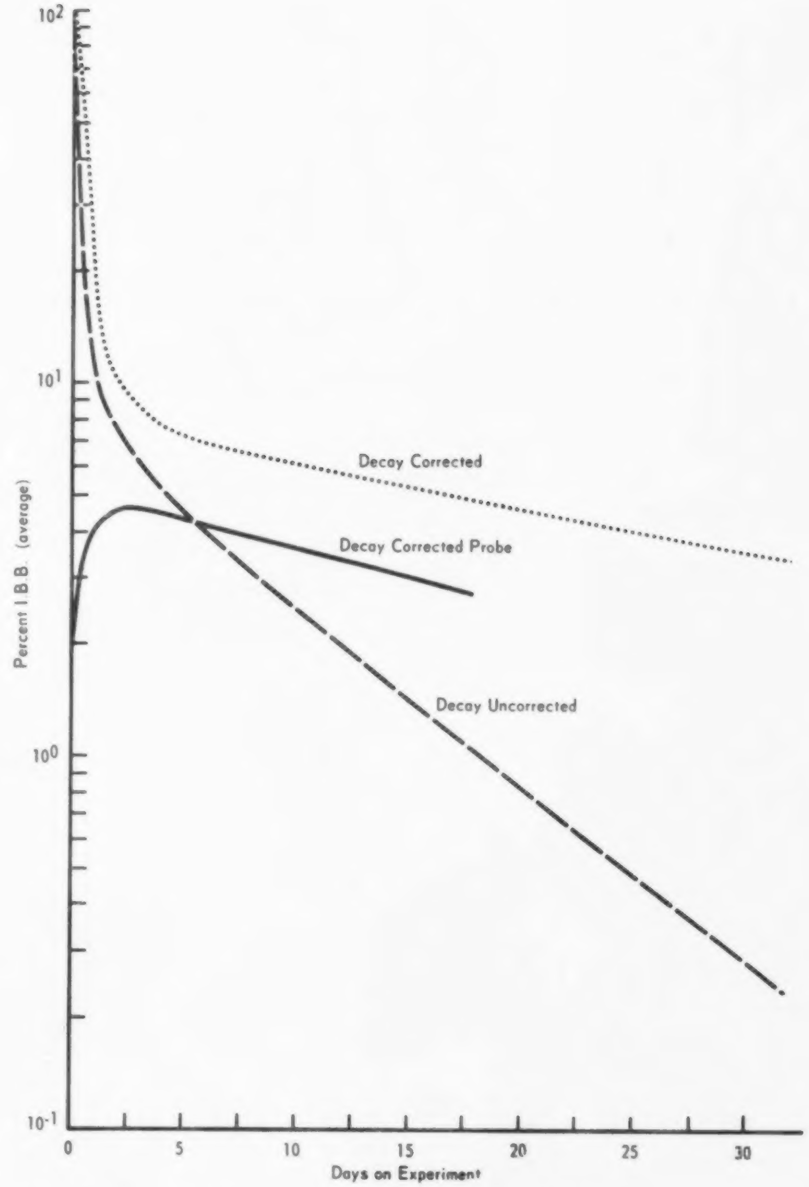


Figure 18. Iodine-131 Retention in Guinea Pigs.

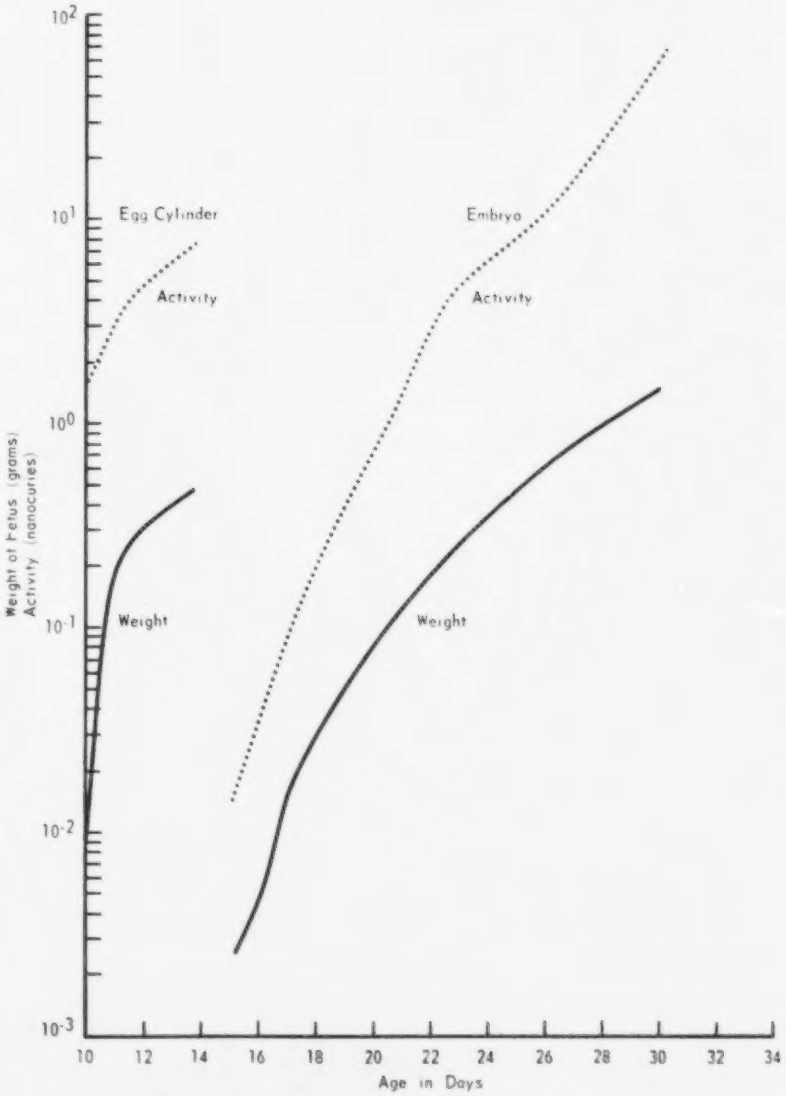


Figure 19. Weights and Iodine-131 Activity in Embryonic Guinea Pigs.

b. Distribution and Teratogenic Effects of Iodine 131 in Embryonic Guinea Pigs

R. M. Hoar, N. S. Nelson, J. F. Stara, A. H. Wolff

Autoradiographic examination of the deposition of radioiodine within the various tissues of the developing embryo is in its final stages. Although dosimetric measurements have not been conducted as yet, preliminary results indicate that during the proliferative stage of development (i.e., rapid cellular multiplication), all tissues concentrate some radioiodine. However, as differentiation and organogenesis progress, most tissues lose their radioactivity, seemingly in direct proportion to their increasing maturation or decreasing proliferation. After extensive trials with various techniques, the freeze-drying method was chosen to prepare sectioned material for autoradiography, because that method results in a minimal translocation and loss of iodine 131 from the embryonic tissue fluids.

As a part of the investigation of the teratogenic effects of iodine 131, 50 pregnant guinea pigs are presently being studied. The pregnant guinea pigs receive chronic doses of iodine 131 during predetermined periods of pregnancy. Observations for teratology are being made on 30-day embryos and neonates, in equal numbers. A number of animals have already delivered and the oxygen consumption, heart rate, and growth rate of their offspring are being measured. No gross abnormalities have been observed as yet in embryos from iodine-treated females sacrificed at 30 days. Histological examination of these embryos is to be completed. However, the resorption rate of fetuses within this group appears to be some 30% higher than the controls. This agrees well with previous pilot studies which suggested that radioiodine administration produces an increased resorption rate and the presence of a soft tissue injury at term (club feet) with no bone deformation. This condition could not be grossly recognized at the 30th day of gestation.

In the neonate, such gross defects of the extremities have been observed. All four feet were hyperflexed and the forefeet were adducted. In offspring where all four extremities were not affected, at least one deformation was found in each case. Examination of cleared specimens, stained for developing bone, showed no bone defects, leaving the possibility of muscle or tendon injury. Statistical analysis of the data must await the inclusion of all the embryos.

c. Chromatographic Analysis of Selected Maternal and Fetal Guinea Pig Tissues for Iodine-131 Compounds

R. G. Wolfangel, N. S. Nelson, R. M. Hoar

In the study of iodine-131 metabolism, the embryonic guinea pigs, the embryos, placenta, and decidua showed radioisotope concentration in all stages of development. Since the iodine could be present as either free iodide or an iodinated compound (P.B.I.), the separation of predicted iodine-131 containing compounds was initiated using chromatographic methods. The tissue samples, after gross gamma counting, were digested enzymatically, centrifuged, and samples were taken for spotting chromatographic plates. After separation the chromatographic plates were used to make autoradiograms. Both thick-layer 2-dimensional chromatograms on cellulose plates, and thin-layer one-dimensional chromatograms on silica gel plates have been tried. Since the most probable source of iodine-131 activity would be either free iodide, iodinated thyronines or iodinated tyrosines, solvent systems to separate these chemical species have been investigated.

Both chromatographic methods have given good separation of five compounds: iodotyrosine, diiodotyrosine, triiodothyronine, thyroxine, and iodide ion. Two unidentified compounds present on the chromatograms will be identified when all autoradiograms are complete.

d. Radioiodine Metabolism in Felines

N. S. Nelson, R. G. Wolfangel, J. F. Stara

Metabolism of iodine 131 in felines was investigated so that it could be compared with that in the guinea pig, and so that iodine-131 metabolism could be compared with the metabolism of radioiodinated tetracycline (see p. 39). Radioiodine, as aqueous sodium iodide 131, was intravenously administered to 12 felines. Radioactivity in whole body and blood were regularly assayed. The retention pattern appears to comprise two independent exponential components. Whole blood displayed the same general downward trend as exhibited by the retention curves.

Of considerable interest was the stepwise function in the iodine-131 blood levels during the first 4 days after iodine-131 dosing. Animals were bled twice daily; the morning samples had in all cases a much higher concentration of radioiodine than the previous afternoon samples. This trend was unmistakably regular and repetitive, and may relate to diurnal cycling. Along with this cycling phenomenon, the feline retained a rather high portion of the initial iodine-131 dose, 80% at 24 hours.

B. RADIOPHARMACEUTICAL METABOLISM STUDIES

1. Repressive Action of Alginates on Strontium Absorption in Cats

J. F. Stara, R. G. Wolfangel, N. S. Nelson

Alginates are long-chain polyuronic acids forming the structural material of many of the brown marine algae. The ratios of guluronic and mannuronic acid units are partially characteristic of the species of algae from which they are derived. There is evidence that the mannuronic to guluronic ratio (M/G) is one of the determining factors for the effectiveness of alginate repression of strontium absorption; an M/G approaching 1.0 produces the greatest inhibition of strontium absorption.

Using cats whose normal diets were supplemented by sodium alginate, calcium alginate, or sodium alginate degraded by hydrolysis, a series of short- and long-term experiments were performed to evaluate the effectiveness of either alginate to reduce strontium in the body, blood, and bone of the cats with respect to calcium levels. Short-term experiments were routinely conducted by administering a single dose of a mixture of calcium 47 and strontium 85 to alginate-fed felines either orally or intravenously. Appropriate control animals, which received a non-alginate diet, were included for each experiment. Experimental observations recorded for the cats included 1) whole-body counting to determine the body burdens of both the strontium and calcium radionuclides, 2) strontium and calcium levels in the blood and plasma, and 3) strontium and calcium levels in selected bones and tissues obtained at sacrifice (both for the radionuclides and stable elements).

In all cases, our results showed that the retention of radiostrontium (whole body, blood, plasma and bone) was markedly reduced by the addition of alginate to the diet of the animals; the reduction ranged from 50 to 80 percent (Figure 20). A slight decrease of radiocalcium level in the experimental cats could also be seen.

Oral doses of strontium 85 were given to alginate-fed cats, as well as appropriate controls, for periods up to 300 days. The strontium 85 was administered as a series of successive daily doses of the radionuclide. Again the results of our experiments illustrate the effectiveness of alginate in producing a significant reduction in the body burden of radiostrontium in the felines which were fed alginate (Figure 21).

Of special interest was Dam No. 278 which was given continuous daily doses of strontium for a prolonged time interval (Figure 22) during which she delivered five kittens (litter No. 3). After delivery and weaning of the kittens, the dam was placed on alginate diet and was again bred. The body burden of the subsequent litter (No. 4) was ten-fold lower than that of the litter born when the dam had received no alginate in her diet.

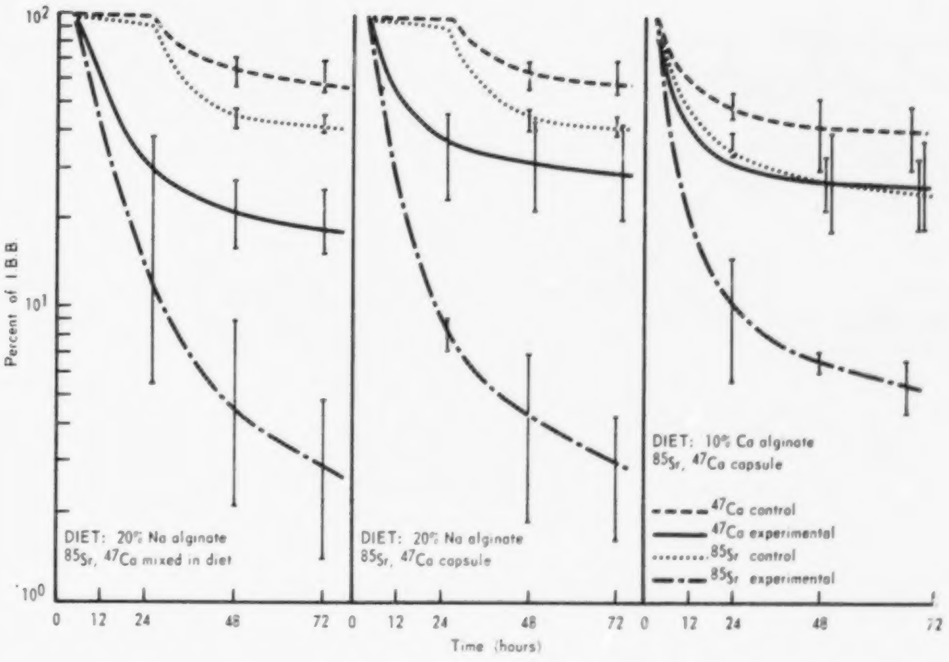


Figure 20. Short-Term Retention of Strontium 85 and Calcium 47 in Cats Fed Sodium or Calcium Alginate.

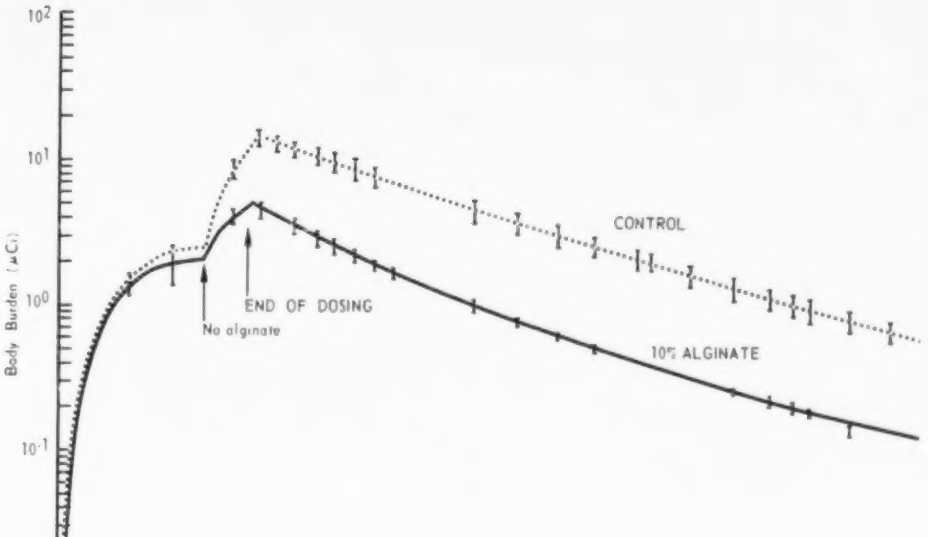


Figure 21. Effect of Sodium Alginate on Strontium-85 Body Burden in Cats (Chronic Dose).

The bones, teeth, and other tissues of a number of cats receiving alginate were analyzed for calcium and strontium. The observations indicated that the observed ratio (Sr:Ca) was inversely related to the duration of alginate feeding.

In summary, an alginate supplement to the diet of felines has effected a significant reduction of radiostrontium levels for whole-body burdens, blood levels, and skeletal levels. The strontium burdens in cats appear to be inversely proportional to the duration of time when the cats were fed an alginate supplemented diet. These were vividly illustrated when short-term studies were performed using calcium 47 and strontium 85, and were confirmed by the marked decrease in the strontium to calcium observed ratios (stable elements) of those cats who were fed alginate for prolonged intervals.

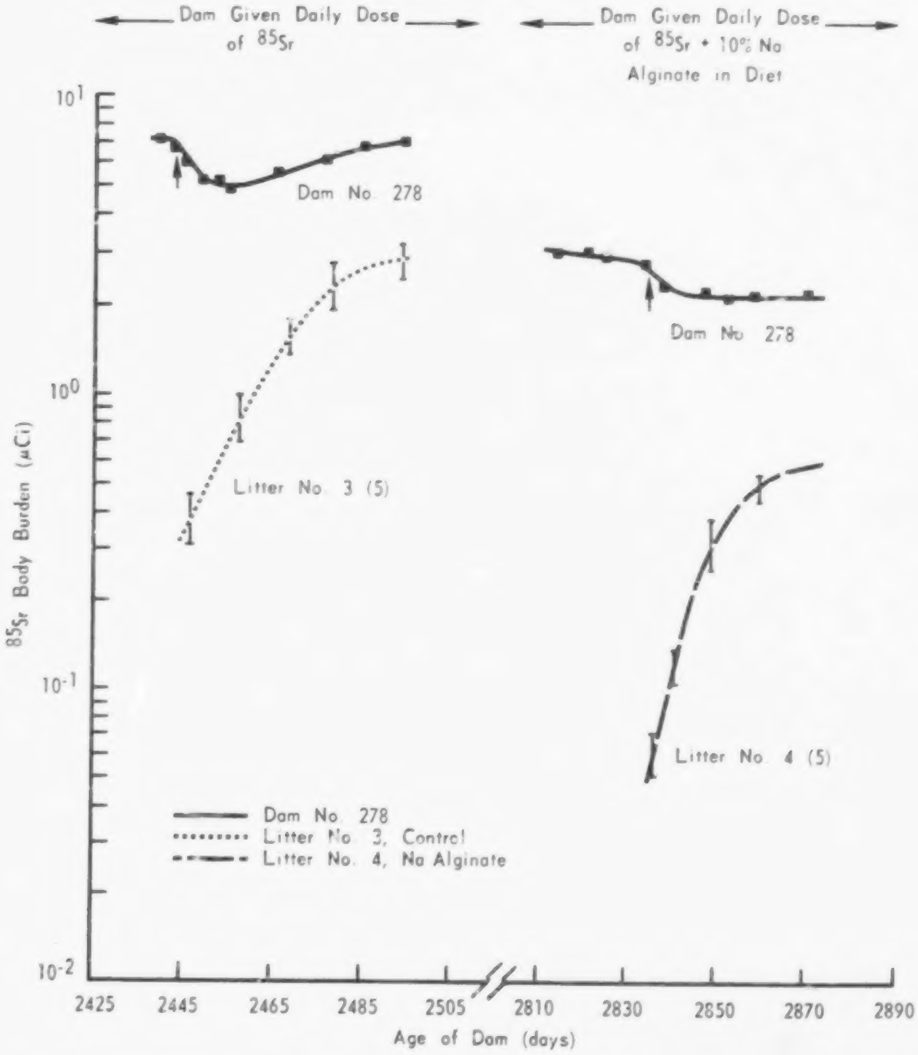


Figure 22. Effect of Alginate in Maternal Diet on Uptake on Strontium 85 in Neonatal Cats.

2. Localization of Labeled Tetracycline and Fibrinogen in Infarcts and Tumors of Mammals

J. F. Stara, R. G. Wolfangel

Three phases of this study were completed: 1) successful iodine-131 labeling of tetracycline, 2) investigations of metabolism of iodine-131 labeled tetracycline in normal felines, and 3) observation of a radioiodinated tetracycline in cats with artifically produced infarcts.

By dissolving 6-demethyl-6-deoxytetracycline in concentrated sulfuric acid with iodine-131 labeled N-iodosuccinimide at $0^{o}C$, we obtained crude 7-radioiodo-6-demethyl-6-deoxytetracycline. Several recrystallizations of the crude material from a 2-methoxyethanol-chloroform mixture produced 99% radiochemically pure labeled tetracycline. Previous studies of this compound in rats, in which excretory products were analyzed chromatographically, demonstrated that this labeled tetracycline was essentially unmetabolized by the rats.

In Figure 23, the retention parameters of iodine-131 labeled tetracycline in the cat are described after intravenous and oral administration. Both groups of animals showed a similar retention pattern; however, total I.V. retention was almost three times higher than in the animals that received an oral dose.

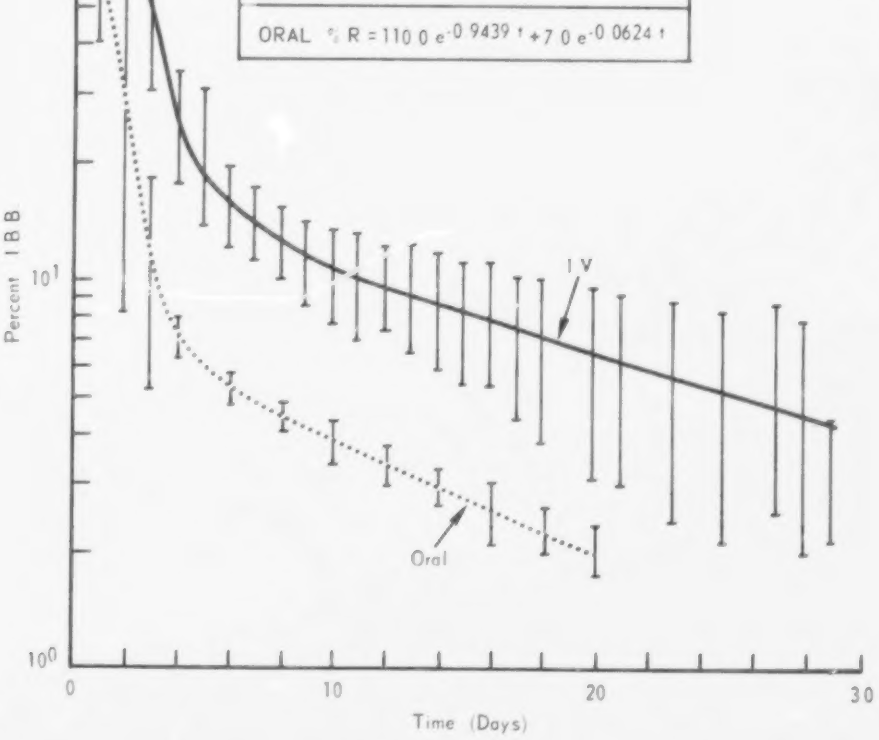


Figure 23. Retention of Iodine-131 Labeled Tetracycline in Felines.

The blood kinetics follow a pattern similar to whole-body retention, except for the presence of the short rapid component in the blood curve of the oral group.

Excretion data plotted in Figure 24, showed an irregular relationship between fecal and urinary values during the first week. Later both routes followed a similar pattern with a F/U ratio of 1.5.

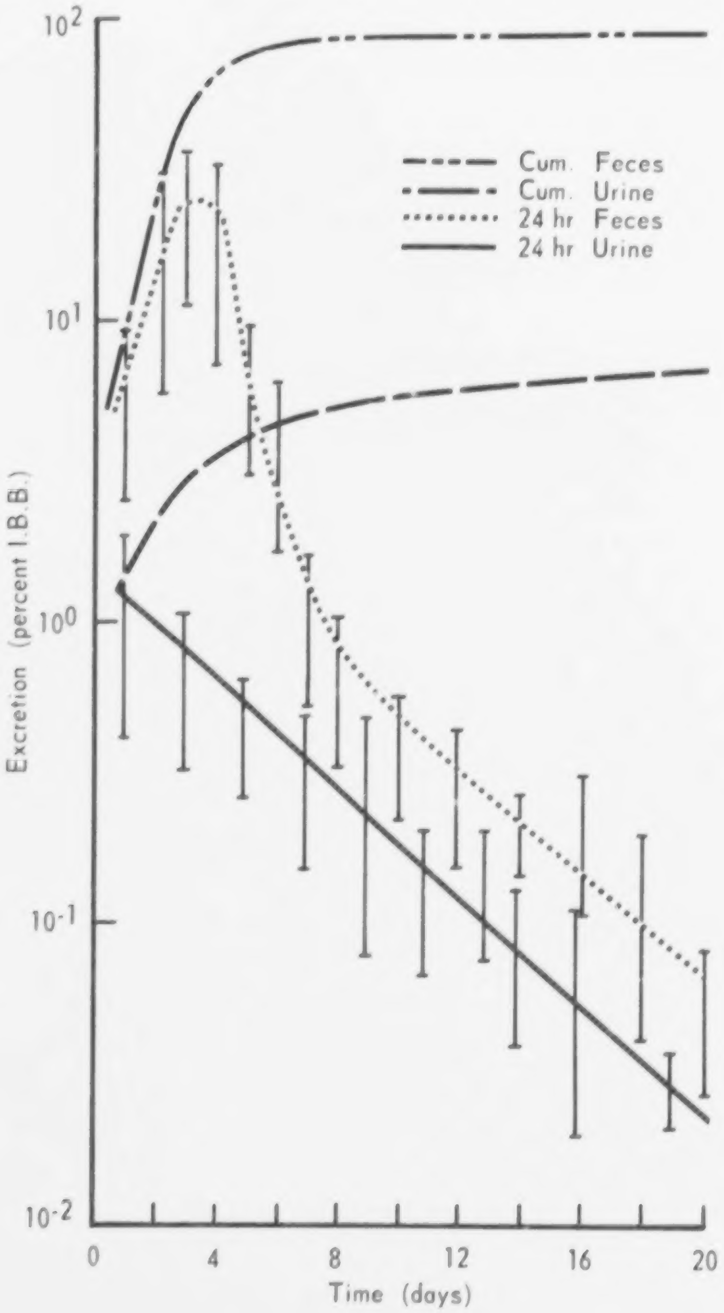


Figure 24. Excretion of Iodine-131 Labeled Tetracycline Intravenously Injected in Cats.

Infarcts were experimentally produced in 20 cats by one of the following methods: arterial ligature, injection of an autologous blood clot, or injection of a commercial grade of fibrin or talc. Later, cats were given a dose ranging from 35-100 mg of iodine-131 labeled tetracycline by intravenous injection. All animals were regularly subjected to whole-body counting, blood sampling, scanning by a scintillation (gamma) camera, and external probe scanning over the infarcted regions. When the infarct was in the femoral region, a slight difference in uptake was noted by external probe scanning; however, this difference was inconclusive using our methods. In addition, the methods used for inducing infarcts produced variable results; therefore the data are difficult to assess and must be further investigated. Whole-body and blood data obtained have been pooled with the values for control animals.

At sacrifice, organ and tissue distribution of iodine-131 labeled tetracycline was analyzed. On a per gram basis, thyroid, bone, gastrointestinal tract, liver and kidney, in that order, were the organs with the highest concentration of iodine-131 labeled tetracycline.

Scinti-scans have proved that radioiodinated tetracycline displayed a definite avidity for the liver and gastrointestinal tract during the first 200 hours after administration. The high tetracycline levels in the abdominal areas obscured the concentrations in the skeleton and infarcted regions. By shielding the abdomen, better details of other areas of the body could be obtained by the scintillation camera, and as a result, smaller differences of iodine-131 deposition can probably be ascertained.

3. Technetium-99m Labeled Albumin in Felines
R. G. Wolfangel

Albumin was synthetically tagged with technetium 99m and introduced intravenously into cats. The cats were whole-body counted regularly and blood samples were obtained at several intervals during the 24 hours following administration. Whole-body retention curves, blood and plasma curves, and tissue concentrations compared favorably to those obtained by other investigators using another species. This comparison indicated that the labeled albumin had been properly prepared in our laboratory. The tagged albumin was originally intended--after thorough animal testing for both sterility and pyrogenicity--for use by the Nuclear Medicine Program for clinical applications involving lung and placental diagnostic scintillation scanning. Curtailment of the radiopharmaceutical program support has terminated this project at this point.

C. METABOLISM AND ASSESSMENT OF HEAVY RADIONUCLIDES

1. Dosimetry of Lead 210 and Polonium 210 in Exposed Populations

R. L. Blanchard

Human soft tissue and bone of an unexposed population were investigated for their content of lead 210 and polonium 210. In addition to lead 210, radium-226 and strontium-90 measurements were included in the study of human bone. The average concentrations of polonium 210 and lead 210 found in human soft tissues are given in Table 5.

The highest average concentrations of polonium 210 in soft tissues were 14.5 and 11.3 pCi/kg in the liver and kidney respectively. Average concentrations in the gonads, lung, and thyroid were approximately one-half as great, and the least polonium 210 was in the spleen, pancreas, and heart. Only in the lung was there a significant difference in the concentration of polonium 210 between smokers and nonsmokers (P=0.001).

Assuming a uniform distribution of polonium 210 within the tissue and an RBE of 10 for alpha particles, the dose-rate in mrems/year is numerically equivalent to the concentration in pCi/kg. The dose rate in the gonads and lung was calculated to be about 7 and 5 mrem/year, respectively, or about half that of the liver and kidney.

The polonium 210:lead 210 activity ratio varied from less than one to greater than one in all tissue types except the lung, in which the ratio varied from 0.5 - 1.0. The greatest amount of unsupported polonium 210 was found to be in the kidney, for which the activity ratio varied from 0.8 to 7.0. In all but two cases, the activity ratio found in the liver was near unity. The occurrence of unsupported polonium 210 found in some human tissues cannot be explained on the basis of present knowledge. This is illustrated in the large difference found between ICRP and measured values of the polonium 210:lead 210 ratio seen in Table 5. A great deal more information is needed to describe lead-210 and polonium-210 dietary intake, and the movement and redistribution of polonium 210 in the human body.

A linear correlation was observed between lead 210 and strontium 90 in human bone. The linear correlation coefficients calculated for lead 210 and strontium 90 in the two age groups investigated, 2 days to 1 year and 4 to 23 years, were 0.89 and 0.67, respectively. No correlation was observed between radium 226 and lead 210. The dose rate delivered to the bones by the polonium-210 lead-210 chain was calculated to be more than twice that delivered by radium 226 plus 30% of its daughters and 3.4 times that delivered by strontium 90.

Epidemiological studies conducted during the past 15 years have shown a very high lung cancer incidence among uranium miners, apparently induced by the radioactive decay of radon daughter products in the respiratory system. Since animal experimentation has failed to disclose any deleterious effects caused by the inhalation of radon daughter products, the uranium miner constitutes the only population known in which an effect (lung cancer) due to radon daughter inhalation is defined. Consequently, a study was initiated during FY 1966 and continued through FY 1967 using this population to investigate various radiological aspects of radon daughter inhalation.

Through FY 1967, soft tissue and bone samples from 22 deceased uranium miners have been analyzed for lead 210 and polonium 210. In some cases, levels of lead 210 and polonium 210 observed in tissues of uranium miners exceeded those normally observed in tissues from unexposed populations by more than 150 times. Adjusting the skeletal levels for excretion of lead 210 during the time interval between exposure and death, a correlation was observed between the log of the lead-210 concentration and the log of the miners estimated cumulative exposure, $R=0.92$ ($P<0.001$). The existence of a possible correlation between lead 210 in the skeleton and in blood is being investigated; however, the data obtained at present are insufficient for any conclusions.

TABLE 5. MEAN CONCENTRATION OF ^{210}Po AND ^{210}Pb IN HUMAN SOFT TISSUES

Tissue	No. of Specimens	^{210}Po, pCi/kg	^{210}Pb, pCi/kg	^{210}Po/^{210}Pb	
				Observed	F Values (ICRP)
Liver	17	14.5 ± 2.0[a]	9.2 ± 1.1	1.9 ± 0.3	0.18
Kidney	18	11.3 ± 2.2	4.3 ± 0.7	2.9 ± 0.4	0.18
Gonads	5	6.9 ± 1.0	7.9 ± 1.2	0.98 ± 0.15	-
Lung	7	5.1 ± 1.4	6.4 ± 1.9	0.88 ± 0.14	0.44
Thyroid	7	5.4 ± 1.6	7.7 ± 1.9	0.69 ± 0.09	
Spleen	19	3.5 ± 0.3	3.7 ± 0.6	1.31 ± 0.22	
Pancreas	17	2.9 ± 0.3	2.8 ± 0.4	1.25 ± 0.20	
Heart	2	0.5 ± 0.1	0.4 ± 0.2	1.50 ± 0.50	

a. Errors are one standard error of the mean

2. Cesium 137 and Natural Radioactivity in the Alaskan Food Chain

R. L. Blanchard

The concentrations of lead 210, polonium 210, radium 226 and cesium 137 were determined in bone, muscle, and rumen content of caribou and reindeer from 15 Alaskan locations in order to gain additional information on the radionuclide content in the Alaskan Eskimo food chain. The polonium-210 and cesium-137 concentrations in the muscle varied with the season, reaching a maximum in the spring and a minimum in the summer. As shown in Table 6, a similar variation in the polonium-210 and cesium-137 concentrations was observed in the rument content. The polonium-210:lead-210 activity ratio in muscle also varied during the year reaching a maximum in the spring. The lead-210 content of the bone did not appear to vary with the season; however, an increase was found with increasing latitude and age. The specific activities of radium 226 in bone and muscle were small in comparison with the concentrations of polonium 210, lead 210 and cesium 137.

TABLE 6. ^{210}Po, ^{210}Pb, AND ^{137}Cs IN REINDEER MUSCLE AND RUMEN CONTENT FROM NUNIVAK ISLAND, ALASKA

Season	Muscle, pCi/kg			Rumen Content, pCi/gram		
	^{210}Po	^{210}Pb	^{137}Cs	^{210}Po	^{210}Pb	^{137}Cs
Fall (Oct.)	135	7.5	8,300	-	-	-
Winter (Jan.)	257	10	28,600	16.7	20.6	30.0
Spring (Apr.)	507	7.1	38,100	25.8	28.7	70.0
Summer (Aug.)	58	15	6,700	4.5	5.2	13.1

It is estimated that adults in the conterminous United States ingest about 2 pCi lead 210/day, less than 2 pCi polonium 210/day, and less than 100 pCi cesium 137/day. In comparison, it was estimated from the results of this study that an adult Eskimo at Ancktuvuk Pass may ingest on the average approximately 11 pCi lead 210, 200 pCi polonium 210, and 12 nCi cesium 137 each day. From these data, it was estimated that the dose contributed by polonium 210 relative to cesium 137 would be about 0.50. That is, an Eskimo who has the maximum body burden of cesium 137 would be overexposed by about 50% due to the presence of polonium 210.

3. Translocation of Polonium 210 from Deposited Lead 210 in Felines

R. L. Blanchard, J. F. Stara, N. S. Nelson

A pilot study was initiated to determine the feasibility of assessing the amount of polonium 210 which is translocated from deposited lead 210 to soft tissue in comparison to the amount remaining in the skeleton.

When lead 210 and polonium 210 enter the body, the lead 210 is concentrated mainly in the skeleton while the polonium 210 concentrates in various soft tissues. This study was undertaken because the polonium 210 present in human soft tissues, including that which is not derived from the lead-210 parent in a particular organ, is derived either by direct uptake from the diet or by redistribution of the polonium 210 present in the skeleton which was formed there by the decay of the lead 210. The mechanics of the movement and translocation of polonium 210 in the body is not presently known, and which of these two sources of polonium 210 predominates is debatable.

As a part of the pilot study, three cats were intravenously administered 0.427μCi of radiochemically pure lead 210. The cats were then sacrificed after 1, 2, and 4 weeks (Table 7), and a distribution study on selected tissues was conducted.

TABLE 7. TISSUE DISTRIBUTION OF LEAD 210 AND POLONIUM 210 IN CATS

	Lead 210												Polonium 210		
	% I.D.[a]/Organ			% S.B.B.[b]/Organ			% I.D./Gram			% S.B.B./Gram			% S.B.B./Gram		
Tissue	1 Week	2 Week	4 Week	1 Week	2 Week	4 Week	1 Week	2 Week	4 Week	1 Week	2 Week	4 Week	1 Week	2 Week	4 Week
Bone	39.7	37.4	22.6	56.76	71.59	82.32	0.130	0.089	0.065	0.186	0.170	0.236	0.000	0.000	0.076
Liver	14.8	8.59	2.58	21.16	16.41	9.40	.182	.059	.022	.261	.115	.078	0.697	.000	.146
Kidney	12.5	2.7	1.29	17.84	5.10	4.70	.471	.072	.039	.675	.138	.142	1.21	.528	.289
Lung	0.190	0.188	0.084	0.271	0.358	0.308	.010	.004	.002	.014	.009	.008	0.029	.145	.056
Muscle	0.758	1.17	0.334	1.08	3.57	1.22	.0005	.0006	.0002	.0007	.0008	.0007	0.0005	.000	.005
Blood	2.02	1.57	0.559	2.88	2.98	2.04	.009	.005	.002	.013	.010	.008	0.038	.252	.139
Urine	-	-	-	-	-	-	.097	.022	.053	.139	.042	.192	0.219	.000	.128
Feces	-	-	-	-	-	-	.043	.030	.049	.062	.058	.179	0.065	.773	.107

a. Initial dose.
b. Sacrificed body burden.

The cat which was sacrificed 2 weeks after injection developed diarrhea and polyuria during the latter part of the second week. This condition, however, was not attributable to the effect of the injected lead 210. The values are included since they follow the general trend; however, those related to soft tissue and excreta may be affected.

The results are listed in Table 7 as percent of initial dose and sacrificed body burden per organ and per gram in case of lead 210; polonium-210 values are shown as percent of sacrificed body burden per gram of tissue. Even though the polonium-210 values are scanty because sufficient time was not allowed in the pilot study for ingrowth from the lead 210, an increased concentration was observed in the skeleton and several other tissues at 4 weeks.

The lead-210 data are more reliable because of the high concentrations of the nuclide in all tissues analyzed. The results show that the skeleton is the major reservoir of lead 210 by the increasing percentage of retention with time, both on organ and gram basis. In general, the soft tissue concentrations decreased at approximately the same rate, and the levels in blood are relatively constant, reflecting the percentage of activity remaining at each time.

This pilot study indicates the feasibility of determining the polonium-210 translocation kinetics, and an experiment clarifying this question is planned.

In addition an uptake, retention, and distribution study in guinea pigs and cats using naturally chemically found polonium-210 compounds (lichen and caribou meat) is in progress.

D. POLONIUM IN TOBACCO AND TOBACCO SMOKE

S. C. Black

The investigation of heavy radionuclides in mainstream tobacco smoke began at the Southwestern Radiological Health Laboratory (SWRHL), Las Vegas, Nevada, in March 1965. The project was a direct consequence of Radford and Hunt's paper[1] on the presence of polonium 210 in the mainstream smoke of cigarettes. Phase I of our study consisted of determining the amount of polonium 210, lead 210 and radium 226 in tobacco and mainstream smoke over a wide range of parameters, i.e., locale of tobacco growth, variability in smoking patterns, effectiveness of commercial cigarette filters, etc. If the exposure to smokers could not easily be eliminated or significantly reduced as suggested by such a parameter evaluation, then Phase II (animal studies to determine whether the amounts of radiation derived from Phase I actually produce lung carcinoma) was to proceed.

The laboratory work on Phase I has been completed and partial results are summarized in the statements that follow.

1) Lead 210, polonium 210, and radium 226 are in approximate equilibrium in tobacco. However, lead 210 and radium 226 in mainstream smoke each contribute only 1/10 as much activity as polonium 210.
2) For a given number of puffs per cigarette, the total amount of polonium as well as its concentration in mainstream smoke increases significantly with the size of an individual puff.
3) Polonium released by the burning tobacco is not readily removed from the smoke by the tobacco the smoke moves through. At best, commercial cigarette filters of various types remove only half the polonium.
4) Based on very limited data, there is an apparent correlation between average polonium levels in a nation's cigarettes and its lung cancer rate.
5) A subject smoking two packs per day of American cigarettes receives an alpha dose-rate to his bronchial tissue which is somewhere between 0.1 and 100 times the alpha dose-rate delivered by the short-lived daughter products of radon at normal atmospheric levels.
6) The alpha dose-rate to the lung tissue can be reduced by selecting a brand with a low polonium-210 concentration, and by adding a reliable filter.
7) Ion-exchange resin can be used as a filter to significantly reduce the amount of polonium 210 in mainstream cigarette smoke.

---

[1] Radford, E. P. and Hunt, V. R. Polonium-210: A volatile radioelement in cigarettes, Science 143:247-249, 1964.

III. RADIATION ONCOLOGY AND PATHOLOGY LABORATORY

N. C. Telles, Chief

The Radiation Oncology and Pathology Laboratory is presently studying some of the pathogenetic mechanisms involved in radiation-induced tumors. The approaches include morphological techniques, biochemical assays, and studies of host-virus relationships in irradiated cells. It is hoped that suitable end points for studying tumorigenesis will arise from these studies.

For the longer term planning it is expected that the relationship between radiation and viruses (both endogenous and exogenous) will receive increasing attention. In particular, cell culture techniques are currently being developed to examine the influence of radiation as a carcinogen. We expect to extend the radiation-viral studies to solid tissues in the future. Spontaneous, as well as radiation-induced tumors will be studied for viral factors, and efforts to establish transmission of these tumors will be made.

A. RADIATION-ETHIONINE ULTRASTRUCTURE STUDIES

N. C. Telles, D. W. Coble

We are currently assessing the possible synergism between whole-body X radiation and ethionine added to the diet and fed continuously to rats (see p. 54). Several hepatomas have now developed and a preliminary morphological evaluation of these hepatomas has been completed. A rather unusual finding has been noted in varying degrees in the hepatomas examined thus far. This is the phenomenon of nuclear invagination.

Phagocytosis of foreign substances by the cell membrane as well as autophagocytosis are well-known phenomena. Phagocytosis by the nuclear membrane has only occasionally been reported, although inclusion bodies seen in cultured cells as well as intact tissues, could in many instances be deduced as deriving from an endocytotic process.

As an interim report we are presenting some of our preliminary findings of this phenomenon which we have noted in our hepatomas.

Random-bred female Osborne-Mendel rats weighing between 120 and 150 grams were given 200 rads of whole-body radiation. Two weeks later they were placed on a synthetic diet containing 0.25% DL-ethionine. Hepatomas developed 7 to 10 months following radiation. Three hepatomas have been noted in rats given both ethionine and radiation. One hepatoma has been noted in a rat fed ethionine only.

These animals were sacrificed as part of a routine sacrifice schedule, and hepatomas discovered at sacrifice. Portions of the hepatoma and adjacent liver were taken for light and electron microscopy and processed by our routine techniques. The findings are briefly described and illustrated in the accompanying figures.

Figure 25 is an example of a nucleus of a hepatoma cell containing rather extensive amounts of cytoplasmic material. Several such areas are designated at CC, and include such cytoplasmic structures as a Golgi apparatus, endoplasmic reticulum, lipid droplets and a myelin figure. Also within the nucleoplasm of this same nucleus is an area, designated CR, in which are distributed other cytoplasmic elements.

Two early invaginations are seen occurring at the nuclear membrane (IN). There are two nucleoli present containing relatively large amounts of fibrous amorphous material.

Figure 26 is a higher magnification of part of the same nucleus. The nuclear membrane is designated by N, and it appears that an invaginated process has been cut in tangential section within the nuclear substance, clearly showing the pores of the nuclear envelope.

Figure 27 is a high magnification of another nucleus showing an invaginated nuclear membrane forming a vesicle which is apparently in the process of completing its separation from the nuclear envelope. Cytoplasmic contents are readily identified within the nuclear body.

A few comments are in order about these illustrations as well as the phenomenon of nuclear invagination. We can only surmise, based on these and other photographs, that the cytoplasmic inclusion bodies in the nuclei are real. They have been observed by other investigators. However, we are aware that they may be artifacts caused by sectioning, and represent the appearance one would get from nuclei with severely infolded nuclear membranes. We are presently studying serial sections of nuclei to resolve this question. We feel, however, based on our currently available material, that the phenomenon we observe represents true nuclear endocytosis.

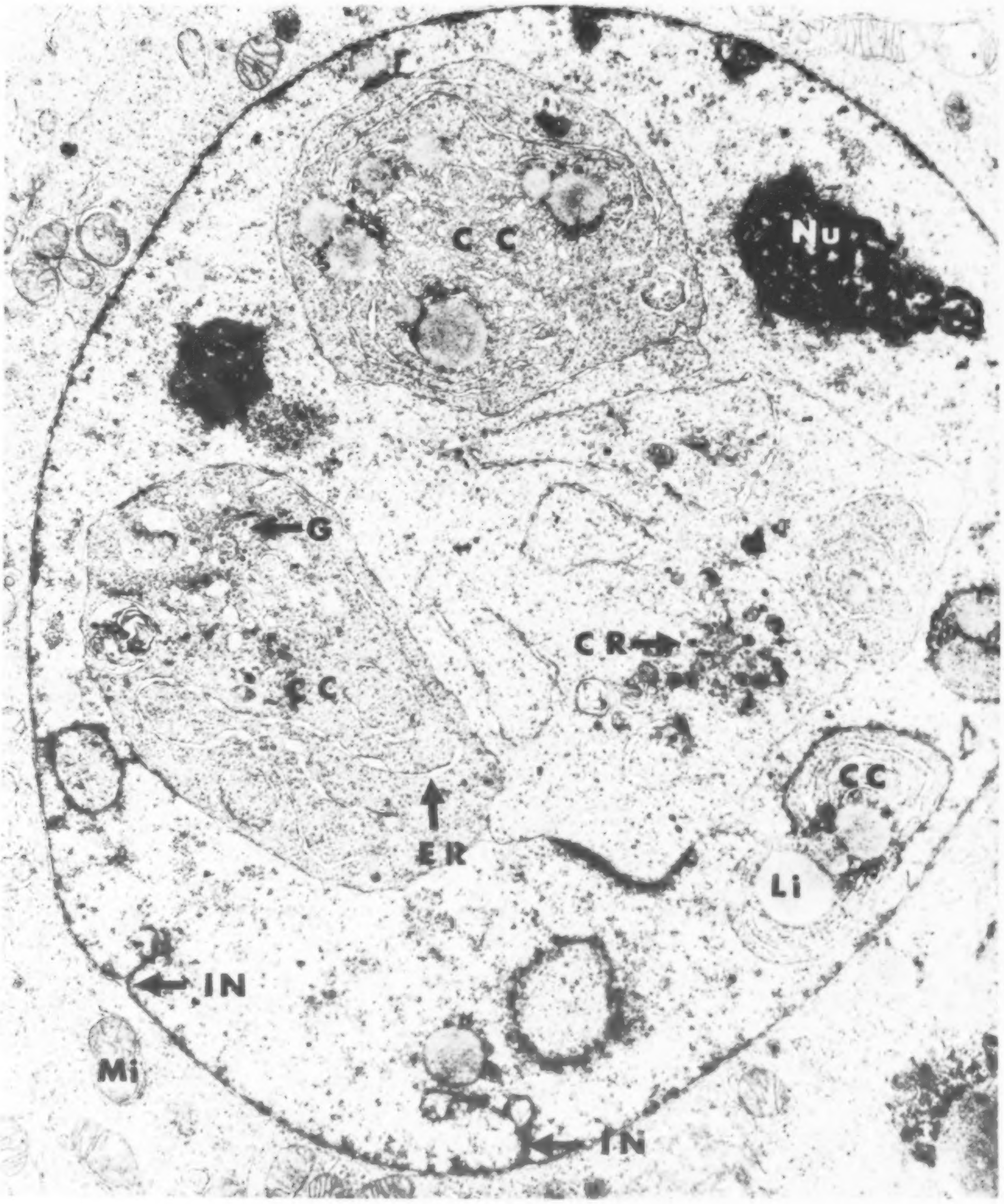


Figure 25. Nucleus from Hepatoma Cell. Large areas of invaginated cytoplasm (CC) occupy a significant fraction of the nuclear section. Cytoplasmic remnants (CR) are seen in the nucleoplasm. Also, endoplasmic reticulum (ER) appears to be present both within the cytosed elements as well as within the nucleoplasm. Invaginating processes (IN) are seen at the nuclear membrane. The nucleoli (Nu) are prominent. Mitochondria (Mi) are small with abnormal cristae in some. Lipid (Li) is seen inside and outside the nucleus. A Golgi apparatus (G) is present within one of the cytoplasmic nuclear inclusions. Magnification X 8,750.

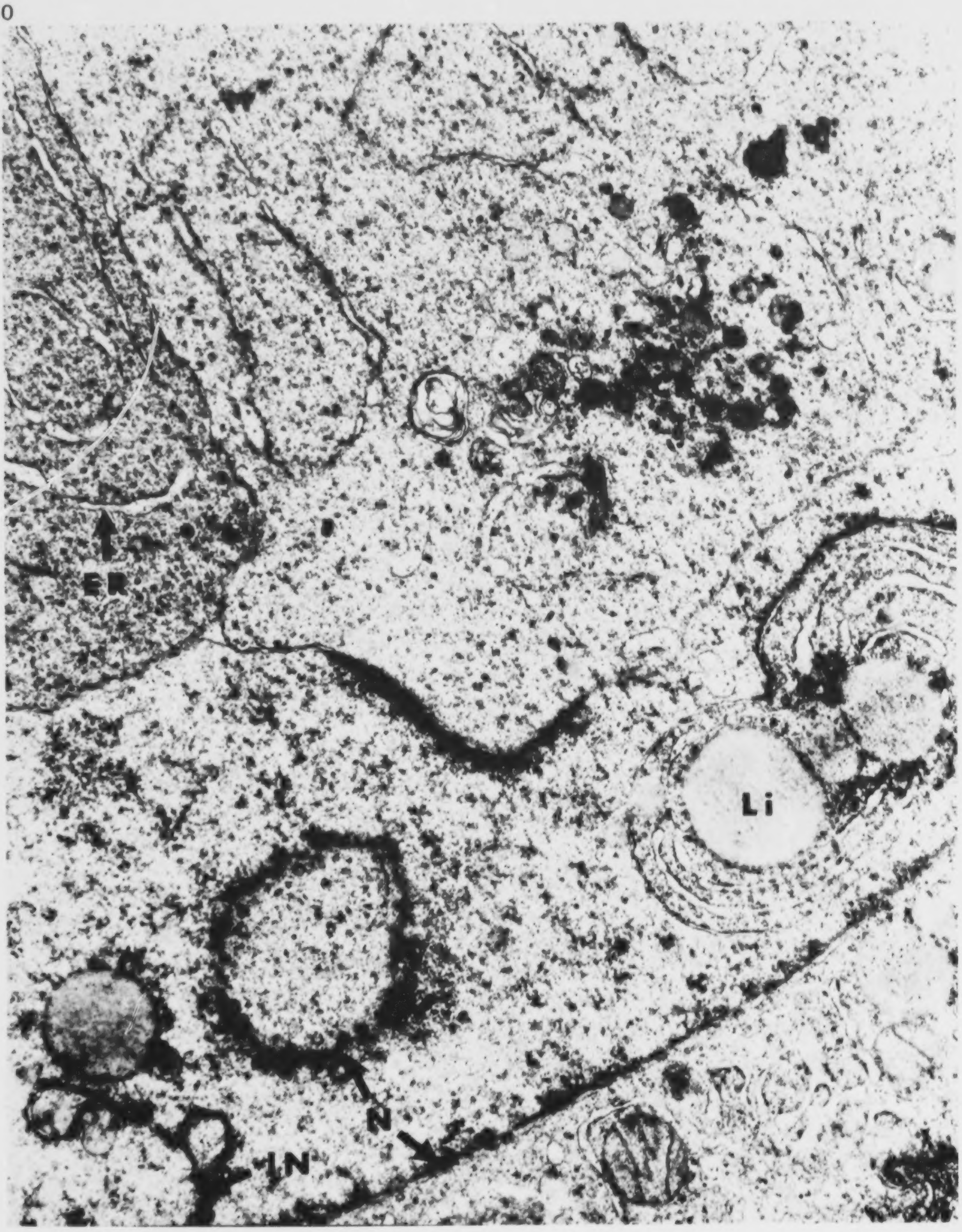


Figure 26. Higher Magnification of Area in Figure 25 Showing Cytoplasmic Remnants (CR) and Invagination of the Nuclear Membrane (IN). The nuclear membrane (N) appears to have been sectioned in two different planes, one at right angles to the normal nuclear envelope, and the other tangentially to what appears to be an invaginated cytoplasmic body. Nuclear pores are clearly seen in the latter. Magnification X 16,000.

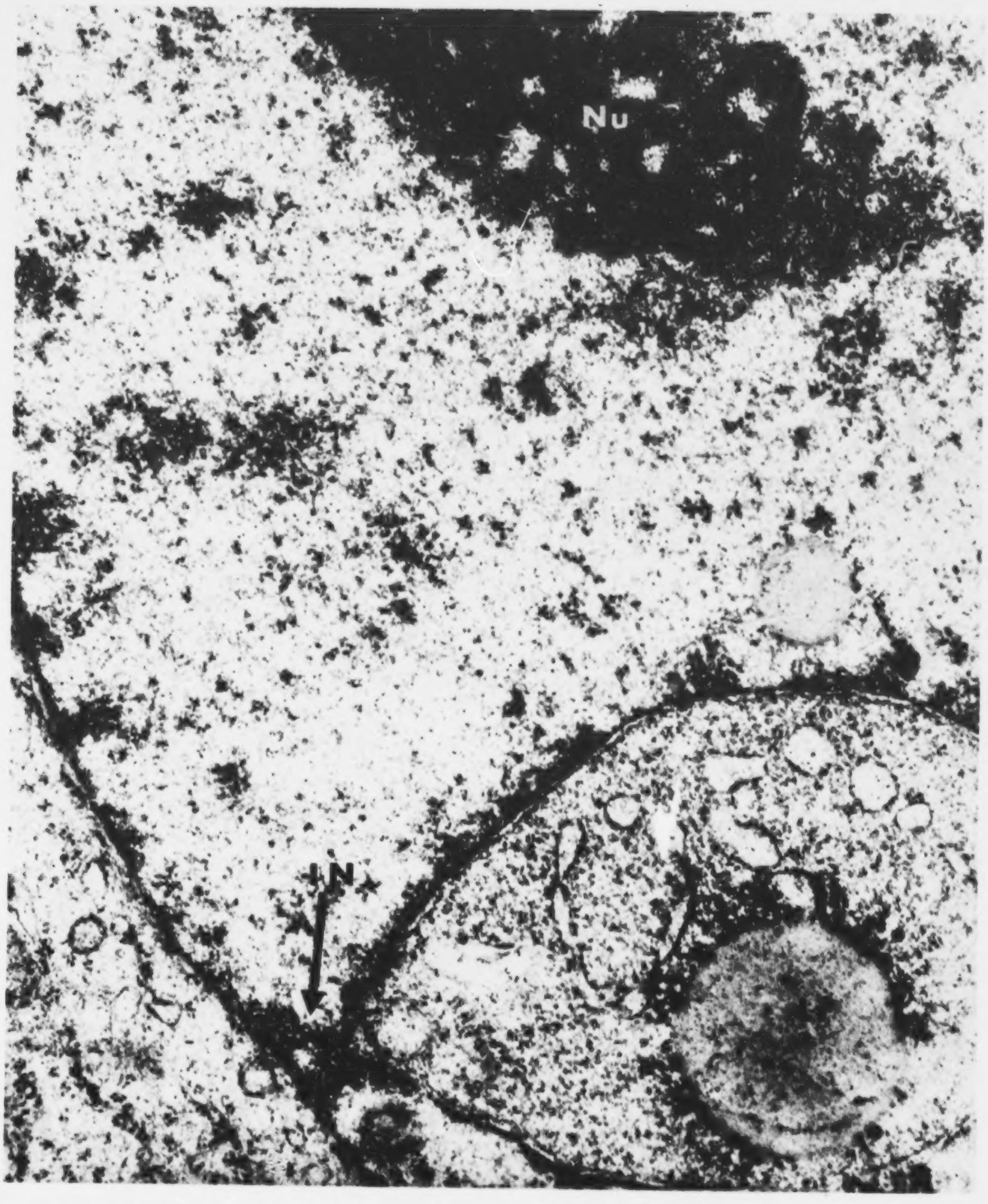


Figure 27. Part of a Nucleus from a Hepatoma Cell Showing the Invagination (IN) of the Nuclear Membrane in What Seems to be the Final Stages of Endocytosis by the Nucleus. There is a band of peripheral nuclear chromatin still joining the nuclear membrane and the cytosed cytoplasm at IN. The section is a little thick but it appears as if the nuclear membrane has reformed. Magnification X 28,000.

B. THYROID TUMORIGENESIS STUDIES

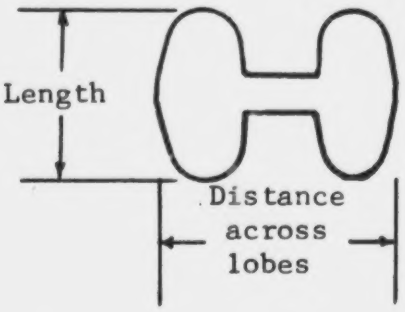
J. R. Childress, T. R. Horton

The thyroid tumorigenesis studies conducted in this Laboratory encompass age sensitivity, dose rate effects, and hormonal effects. The ability of X rays to produce thyroid tumors in rats of different ages is the principal study. Animals at ages of 10 days, and 1, 3, and 6 months are being irradiated and given a common risk period to develop neoplasms. The X-ray beam is collimated by specially designed brass collimators so that only the thyroid will be irradiated, not the pituitary. Thyroid dimensions and thyroid-pituitary measurements for each age group are given in Table 8.

TABLE 8. THYROID DIMENSIONS OF RATS AT DIFFERENT AGES

Age	No. of Rats	Thyroid Dimensions, mm Length	 Distance Across Lobes	Pituitary-Thyroid Distance[a], cm
10 days	3	3	4	0.9 - 1
1 mo.	3	5	6	1.2
3 mo.	3	6	7	1.6
6 mo.	3	6	7	1.8

a. Nearest point of thyroid to pituitary



Since very little is known about the effect of dose rate on the production of thyroid tumors, a dose rate study has been planned. Two different dose rates varying by a factor of 10-30 will be used to obtain the same total dose.

Finally, a study will be undertaken to determine the effect of estrogen on thyroid tumorigenesis. Ten-day-old rats will be irradiated and be given estrogen daily for approximately 70 days. From this experiment we hope to determine whether the age of the animal or the sexual maturity of the animal influences thyroid tumorigenesis.

Our preliminary experiments have involved determining the rad dose to the thyroid and scattered dose to the pituitary by utilizing LiF dosimeters in place of the glands themselves in frozen rats of each age group.

C. HOST-VIRUS RELATIONSHIPS IN IRRADIATED CELLS

E. J. Pollock

In several reported studies a virus or a virus-like principle isolated from radiation-induced tumors has apparently caused similar tumors when transferred to other animals. For studies like those conducted in the Radiation Oncology and Pathology Laboratory, we must establish whether principles such as viruses or infective deoxyribonucleic acid or ribonucleic acid are present in the tumor or the cultured tumor cells. Beside establishing a tumor-virus relationship, such investigation might also provide information useful in the fields of 1) irradiation preservation of foods, 2) irradiation sterilization, or 3) the use of irradiation-inactivated virus in vaccines.

Other investigators[1,2] have found a cell-virus system in which irradiation increases the efficiency of viral transformation in vitro. Most of the irradiated cells in those studies were killed, however, and there was only a relative increase in the fraction of transformed colonies.

Using synchronized cultures of the mouse cell line, 3T3, in our early investigations, we have found recovery conditions that can reduce cell mortality caused by radiation. Thus effects of irradiation on transformation by SV40 virus can now be determined in this system. We also have established a system whereby the same kind of study can be done in diploid human cells.

Reports by others[3,4] have suggested that when tumor viruses are irradiated, infectivity declines faster than oncogenecity. However, the studies of infectivity and oncogenecity were conducted in separate cell systems. It is hypothesized that less genetic material is required to code for transformation than to code for infection, and it is possible that if infectivity can be aborted by irradiation, a virus may still be capable of effecting transformation. We have developed a system where both infectivity and transformability can be studied in the same cell line, a line of diploid human cells. The effect of irradiation on several oncogenic and non-oncogenic viruses is now being studied in this system and should provide data that will be applicable to man.

---

[1] Stoker, M. Effects of X-irradiation on susceptibility of cells to transformation by polyoma virus, Nature 200:756-758, 23 Nov. 1963.

[2] Stoker, M. Further studies on radiation-induced sensitivity of hamster cells to transformation by polyoma virus, Virology 24:123-125, September 1964.

[3] Latarjet, R., Cramer, R., and Montagnier, L. Inactivation by UV-, X-, and γ-radiations of the infecting and transforming capacities of polyoma virus, Virology 33:104-111, 1967.

[4] Defendi, V., Jensen, F. Oncogenicity by DNA tumor viruses: Enchancement after ultraviolet and cobalt-60 radiations, Science 157 (3789):703-705, 11 August 1967.

D. RADIATION-ETHIONINE CO-CARCINOGENESIS STUDY

B. C. Ward, N. C. Telles

This study is designed to establish whether radiation plus ethionine produces more liver tumors in rats than either radiation or ethionine alone. In this phase of the study a single radiation dose was given to the liver area or to the whole body. Four levels of ethionine were fed in a special diet for 18 months.

1. Mortality

Of 279 rats entered in the study, 85 (30%) died before termination of the experiment. As shown in Figure 28, almost 50% of the deaths were related to mammary tumors. This is not an unusual finding in long term studies of rats exposed to ionizing radiation. The number of rats dying from other causes was probably not related to radiation or ethionine. Figure 29 graphically depicts mortality rates for each treatment group.

At the end of 18 months the remaining 194 rats were killed. Body weights and liver weights were taken and samples from various organs were placed in 10% formalin. Results of the histopathologic examination of these organs are given below.

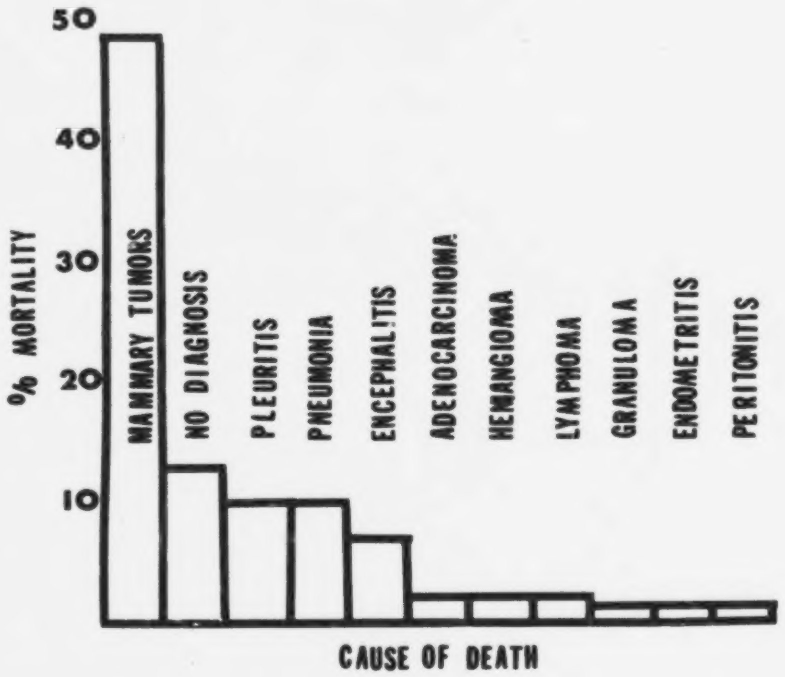


Figure 28. Cause of Death in Irradiated Rats Fed Ethionine.

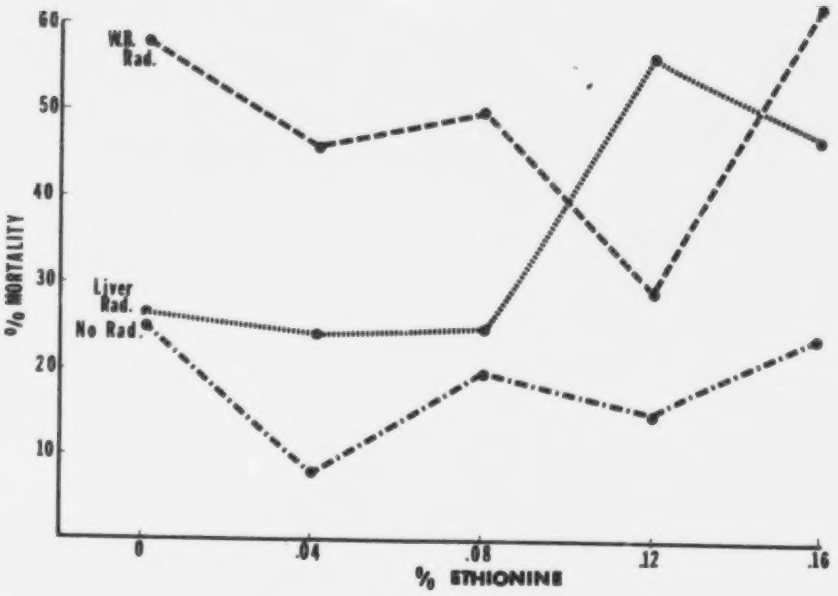


Figure 29. Mortality Rate for Each Treatment Group.

2. Histopathology

Liver Most of the livers from rats fed ethionine exhibited extreme fatty metamorphosis of the hepatic parenchymal cells, hyperplasia of bile ducts, and pericholangitis (Figure 30). Nodular hyperplasia was present only rarely (Figure 31) and no liver tumors were produced with the low ethionine levels fed in this experiment.

Pancreas Atrophy of the pancreas with replacement of the lobules by fat was consistently found in rats fed ethionine and was more pronounced in those animals receiving higher levels (Figure 32).

Brain No changes that could be attributed to ethionine or radiation were found in the brain. One rat had a granulomatous encephalitis of unknown etiology and another had a cholesteatoma.

Pituitary Adenomas were present in 13% of the pituitaries examined.

Thyroid Follicular adenomas were present in 4% of the thyroids examined. Two rats developed carcinomas of the thyroid gland. Hyperplasia of the follicular epithelium was found in 6% of the rats.

Adrenal Adrenal cortical adenomas were present in 6% of those examined.

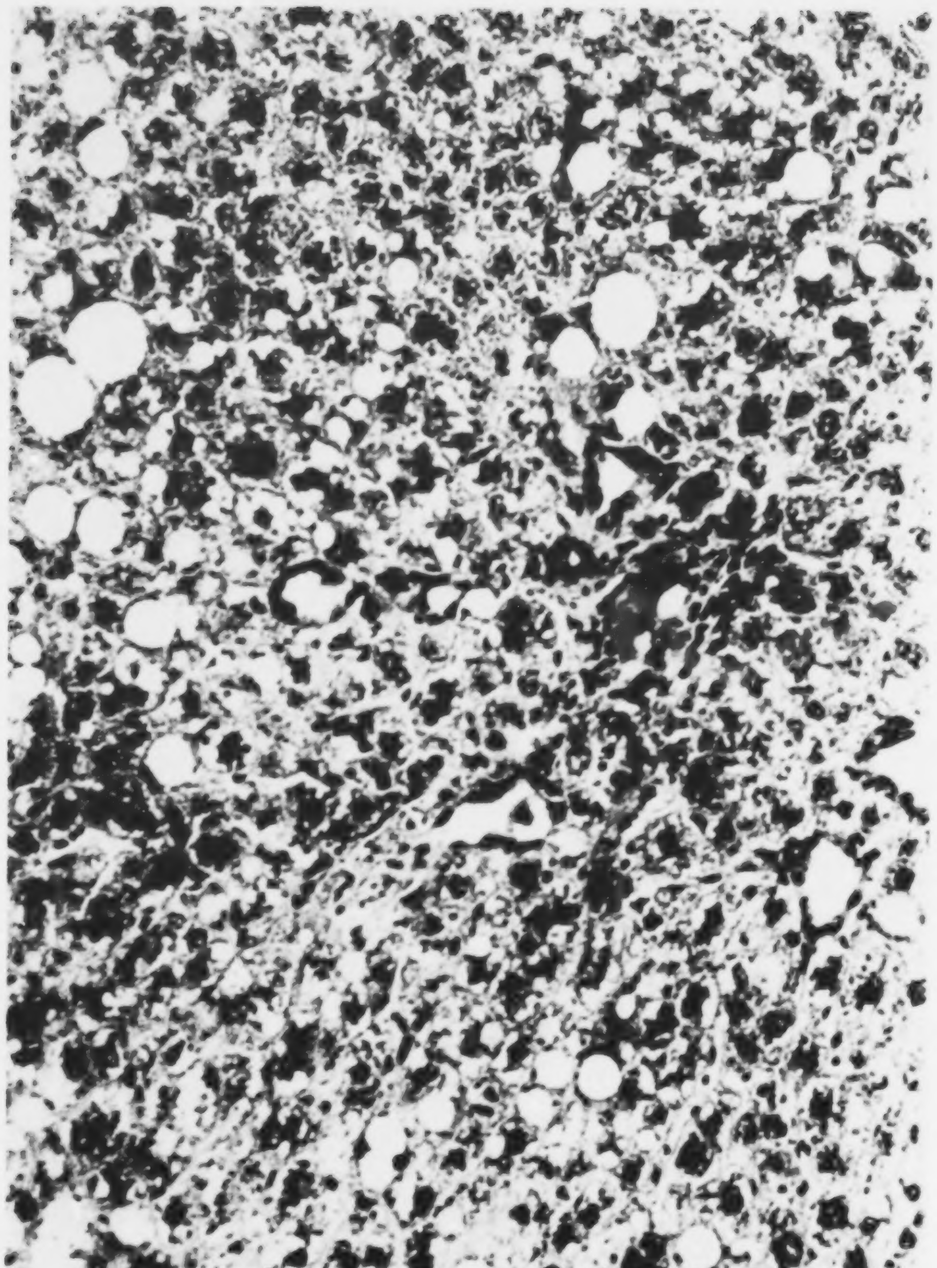


Figure 30. Section of Liver from Rat Fed 0.16% Ethionine for 18 Months. Large vacuoles demonstrate fatty change of hepatic cells. Mild pericholangitis and bile duct proliferation are also present. H & E, magnification X 125.

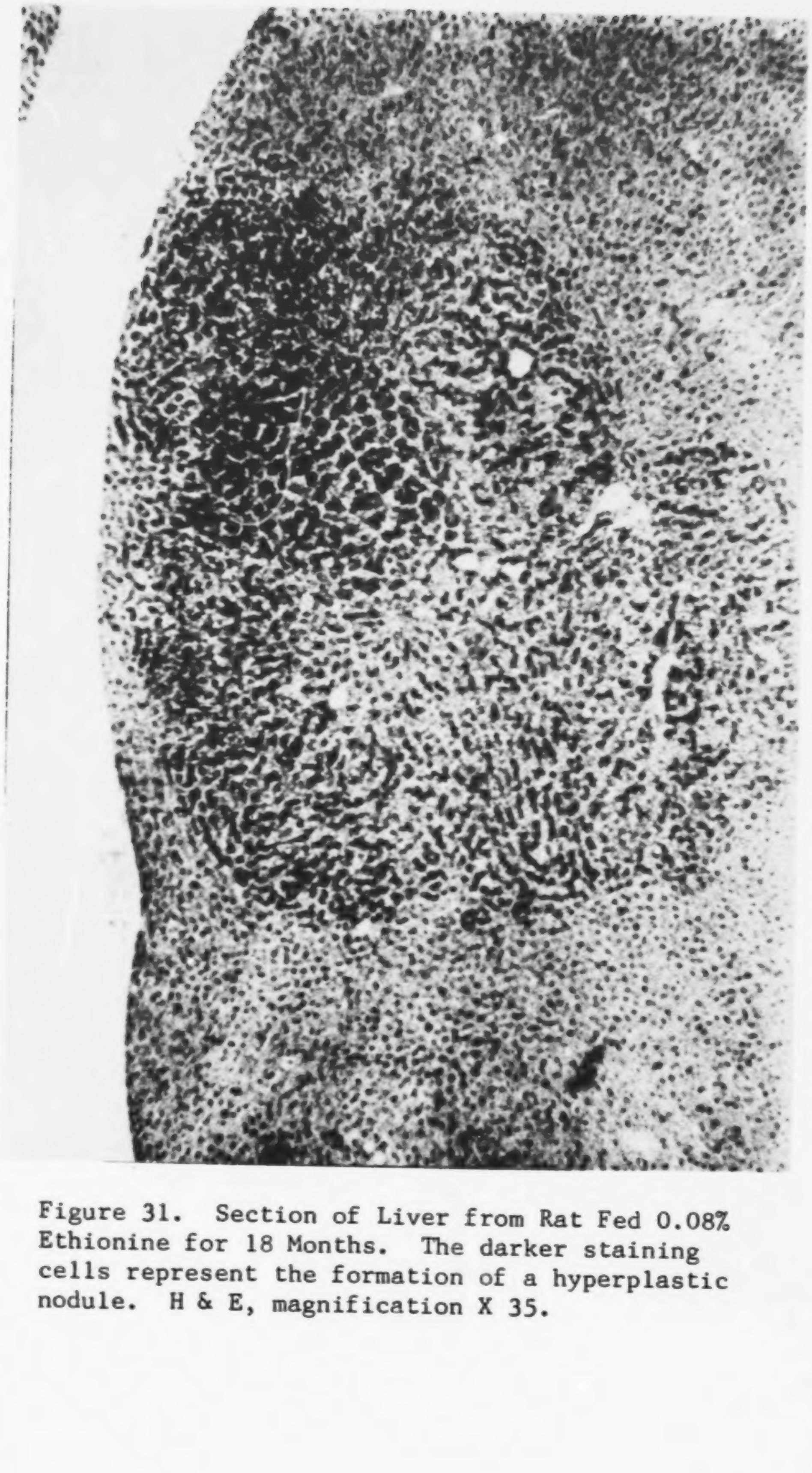


Figure 31. Section of Liver from Rat Fed 0.08% Ethionine for 18 Months. The darker staining cells represent the formation of a hyperplastic nodule. H & E, magnification X 35.

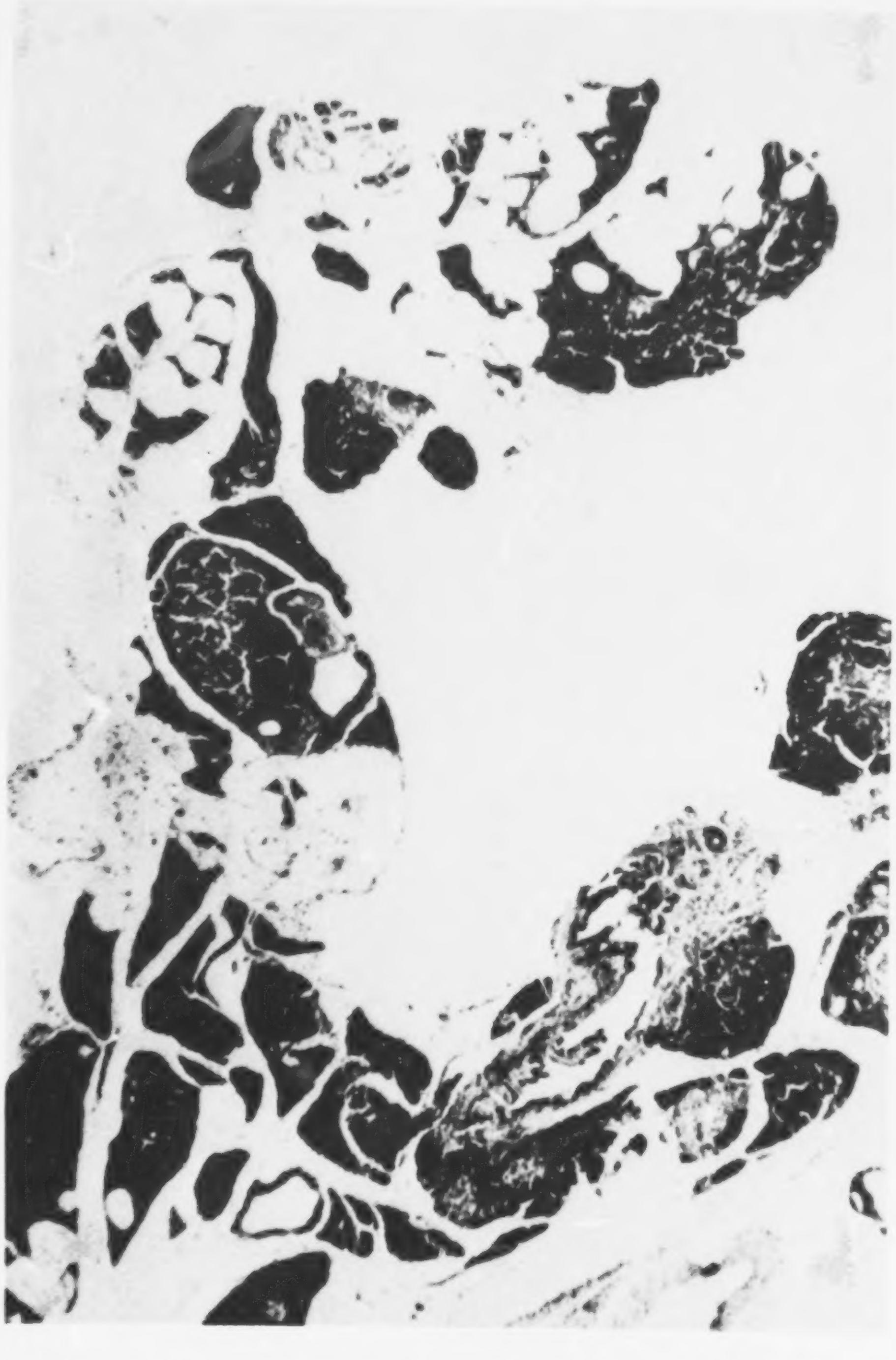


Figure 32. Section of Pancreas from a Rat Fed 0.16% Ethionine Showing Parenchymal Atrophy with Fatty Replacement. Magnification X 35.

Salivary Gland No lesions were found in this organ.

Lymph Node Reactive hyperplasia of the lymphoid elements was present in many of the rats with ulcerated, infected mammary tumors.

Kidney Many of the rats in this experiment exhibited varying degrees of renal changes commonly seen in chronic nephritis in the rat. The kidneys of severely affected animals showed marked tubular dilatation with the presence of proteinaceous casts. Fibrosis was present in some of the kidneys but was not a prominent finding. Glomerular changes were found in many of the end stage kidneys. The renal pathology appeared to be independent of the ethionine or radiation treatment.

Spleen Some of the spleens from rats with ulcerated mammary tumors exhibited reticuloendothelial hyperplasia and, in instances, a leukemoid reaction was observed. One hemangioma was found. No lesions related to ethionine were seen.

Lung Most of the rats showed varying stages of chronic murine pneumonia. Often only lymphocytic peribronchial cuffing was seen but in a few of the rats bronchiectasis and abscessation were found.

Heart Mild focal interstitial myocarditis was present in 3% of the rats. Endocardial fibroelastosis was present in the right ventricle of one of the hearts examined.

Thymus The thymus was examined in only 32 of the rats. One thymoma was present. No other lesions were seen.

Trachea Tracheitis characterized by submucosal infiltration of mononuclear cells was present in 64% of the rats examined. This is a common lesion in rats and is thought to be related to chronic murine pneumonia.

Esophagus No lesions were found in this organ.

Stomach Chronic gastritis was apparent in three of the stomachs examined. One rat had a gastric carcinoma. These changes are probably not related to treatment.

Small Intestine Chronic enteritis was evident in 6% of the rats.

Ovary Eleven granulosa-luteal cell tumors and one papillary cystadenoma were found in the ovaries.

Bone No lesions were found in the bone. Leucocytosis was demonstrated in the marrow of some of the rats with ulcerated mammary tumors.

Uterus Endometritis and endometrial hyperplasia were seen in a few rats and 10% of the uteri examined had polyps.

3. Comment

The results of this study show that rats receiving whole-body radiation and ethionine developed a significantly greater number of mammary tumors than those rats receiving whole-body radiation alone. This increase in tumor incidence appeared to be directly related to the level of ethionine in the diet. However, rats receiving only liver radiation or no radiation showed a decrease in the incidence of mammary tumors when fed increasing levels of ethionine. Figure 33 graphically depicts the relationship of ethionine and radiation to the incidence of mammary tumors. Table 9 gives the type and incidence of tumors seen in this study.

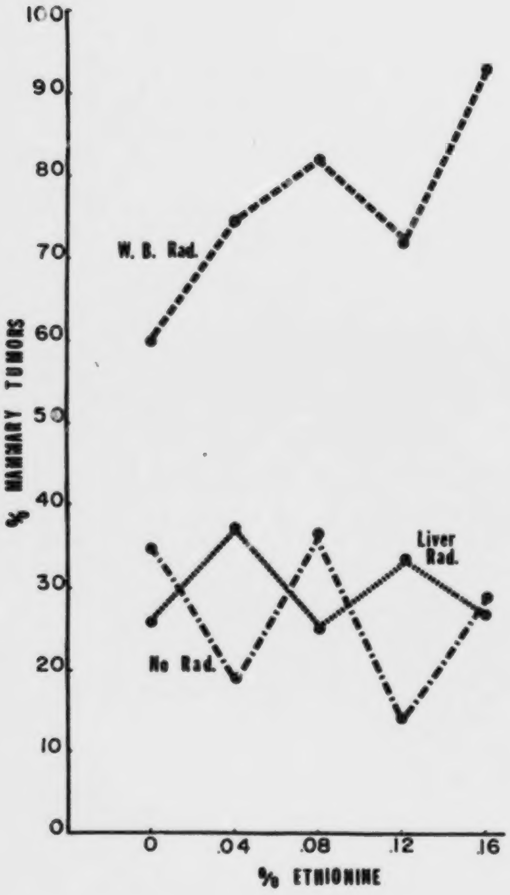


Figure 33. Relationship between Radiation, Ethionine, and Incidence of Mammary Tumors.

TABLE 9. TUMOR INCIDENCE BASED ON 279 RATS IN STUDY

Mammary tumors	103
Fibroadenoma	79
Papillary cystadenoma	24[a]
Parathyroid adenoma	2
Hemangioma	2
Thymoma	1
Intestinal Carcinoma	1
Gastric carcinoma	1
Granulosa luteal tumor	12
Ovarian papillary cystadenoma	1
Pituitary adenoma	18
Thyroid adenoma	6
Thyroid carcinoma	2
Adrenal adenoma	9
Lymphosarcoma	2

a. Three of these rats also had at least one fibroadenoma

E. BIOCHEMICAL ASSAYS OF IRRADIATED CELLS

E. A. Willensky

The Radiation Oncology and Pathology Laboratory has been studying the biochemical changes in liver as it progresses from normal to cancerous. These studies have encompassed both long- and short-term experiments. In the long-term experiments tryptophan pyrrolase (TP) and total nitrogen content of the liver were examined in control and hydrocortisone (HC) injected animals that had previously been treated with various combinations of X irradiation and ethionine. The short-term experiments followed the acute effects of X irradiation and ethionine on TP levels in control, HC, and tryptophan treated animals. Male rats seem to be protected against the acute effects of ethionine such as depression of protein synthesis. Using tryptophan pyrrolase as a model system of protein synthesis, we have studied the effect of pre-treating female rats with testosterone, and have shown that the depression of protein synthesis by ethionine can be partially reversed by testosterone treatment. Quantitation of these effects is now under consideration in our laboratory.

To establish a means for storing a large number of liver samples, the stability of TP was studied in various preparations. The TP (total enzyme) is completely stable at -120°C in the supernatant of the 105,000 G centrifugation for as long as six weeks. However, at the same temperature it was not stable for even 24 hours as an intact liver or as a homogenate. At 2°C the enzyme was not stable in any form. Thus, we have established a method of storing liver samples for extended times without losing total TP activity. A full report is being prepared.

The initial biochemical observations on the hepatomas removed from rats that received X irradiation and ethionine indicate that this tumor has essentially no TP in either base-line or HC treated animals; however, the grossly normal appearing parts of the same livers do possess enzyme and retain the ability to respond to HC. The relationship of this finding to disturbances in intermediary metabolism in cancer cells is under consideration. In this regard, ultrastructure studies of these same hepatoma-bearing livers show very little endoplasmic reticulum, fewer mitochondria and other normal cytoplasmic structures in the hepatoma, although the adjacent liver cells show a considerable amount of each. This may help explain, on a morphological basis, why the hepatomas have low basal levels of TP, and fail to respond to cortisone. In general, it has been found that "minimal deviation hepatomas" respond better to tryptophan or cortisone induction than do less well differentiated hepatomas.

Since TP is the committing enzyme in the conversion of tryptophan to pyridine nucleotides, and since oxidative metabolism may be abnormal in this tumor as it is in many others (Warburg hypothesis), the relationship between pyridine nucleotides, depression of oxidative phosphorylative mechanism, and depression of tryptophan pyrrolase levels (protein syntheses) was studied. We found that in intact animals pyridine nucleotides "induce" TP, an effect that was obliterated by adrenalectomy. This indicated an indirect mechanism of induction. However, the addition of pyridine nucleotides to HC-injected adrenalectomized animals at various times after injection resulted in a significant depression of the induction of TP. Further studies on the elucidation of the mechanism of TP depression in cancer cells are now in progress.

In the past several years, increasing evidence has indicated that DNA and RNA (messenger, ribosomal, and transfer) are present in mitochondria, and that these nucleic acids act in a genetic fashion much like our model for nuclear DNA and RNA. Preliminary experiments are being pursued in the light that acute radiation damage as well as long-term carcinogenic effects may be mediated by a cytoplasmic mitochondrial mechanism. Preliminary data seem to indicate that mitochondrial "disruption" is at least contributory to the acute effects of radiation.

IV. RADIATION PHYSICS LABORATORY

H. D. Youmans, Chief

The Radiation Physics Laboratory provides services to the Radiation Bio-Effects Program and conducts research studies in radiation dosimetry. The services consist of (i) dosimetry and irradiation of all biological specimens, (ii) maintenance of a central tracer-level radioisotope counting facility, and (iii) engineering design and fabrication of nuclear and biomedical laboratory instrumentation. The Radiation Physics Laboratory also conducts research studies both collateral to the biological interests of the Program and of intrinsic value to health physics.

A. LABORATORY SERVICES

1. Irradiations

W. Lee, A. L. Richmond

The irradiation of laboratory specimens during the 1967 calendar year is presented in Table 10. All dosimetry is based upon absorbed dose. Thermoluminescent dosimetry is commonly used with tissue equivalent animal phantoms in designing the irradiations. Preliminary phantom dosimetry, secondary dosimetry for accuracy assurance and animal handling require as many man-hours as the specimen exposure time; thus, this service approximates one-half man-year exclusive of occasional ancillary methods studies. Permanent records are maintained in both a standardized card file and data book.

TABLE 10. X IRRADIATIONS AT THE TWINBROOK LABORATORY, 1967

Specimen	Number	X-Ray On-Time, Hours
Rats	2,538	286.3
Biological Specimens	1,407	35.9
Dogs	37	8.5
Hamsters and Mice	305	129.7

2. Instrumentation

F. C. Pendleton, W. H. Ridgley

Of the numerous contributions of the Biomedical Instrumentation Unit throughout the year, two items have been of special value to the Program. Two rotary rodent exposure chambers have been fabricated from acrylic plastic (Figure 34). Each consists of 24 cells and is designed to allow for non-uniform spatial distribution of intensity characteristic of therapy-type X-ray tubes. Attention to X-ray scattering and rotation about the central axis of the working beam has resulted in the ability to routinely provide class-A irradiations (NBS Handbook 88, ICRU Report 10e, 1962). Identical units make possible simultaneous sham irradiation and exposure, and reduce time delays due to animal handling between irradiations.

Because of the subtle effects of low doses of radiation on the organs of experimental animals, occasionally animals must be sacrificed by other than conventional means. We have fabricated an electrical power unit that can be used to sacrifice rats humanely, but leaves no toxic products in the animals' organs as some chemical agents do. The unit has a variable potential from 0 to 150 volts ac at 1 ampere maximum. Electrical power is fed to a rubber glove that contains one electrical contact on the thumb, and another on the first finger (Figure 35). To sacrifice an animal, the technician wearing the glove merely squeezes an animal gently on either side of the head with his thumb and forefinger.

3. Development of Electronic Circuits

G. E. Anderson, R. A. Tell

As a part of its continuing service to the Radiation Bio-Effects Program, the Radiation Physics Laboratory has developed a number of electronic circuits for detecting and measuring particular radiations or emissions. One of the two discussed here provides a convenient method for quickly locating activity peaks on gamma spectra. The other device, an analog-to-digital converter enables the investigators to obtain additional information from the readout of thermoluminescent dosimeters that was unavailable by the original design of the apparatus.

a. Aural Rate Meter

Often the location of activity peaks in gamma spectra is tedious and slow. Elsewhere, it has been found that an aural rate meter can be used to advantage. Previous designs have been based upon vacuum tube components which require undesirable bulk, weight, and power. To overcome these difficulties, it was determined that an audio oscillator, whose frequency would vary in proportion to the input pulse rate, would be ideal to locate the peaks. If the pulse rates taken from a single channel analyzer window were fed into the circuit, the operator could "tune in" the spectrum peaks by ear, merely by moving the analyzer window and listening for a peak in the oscillator frequency.

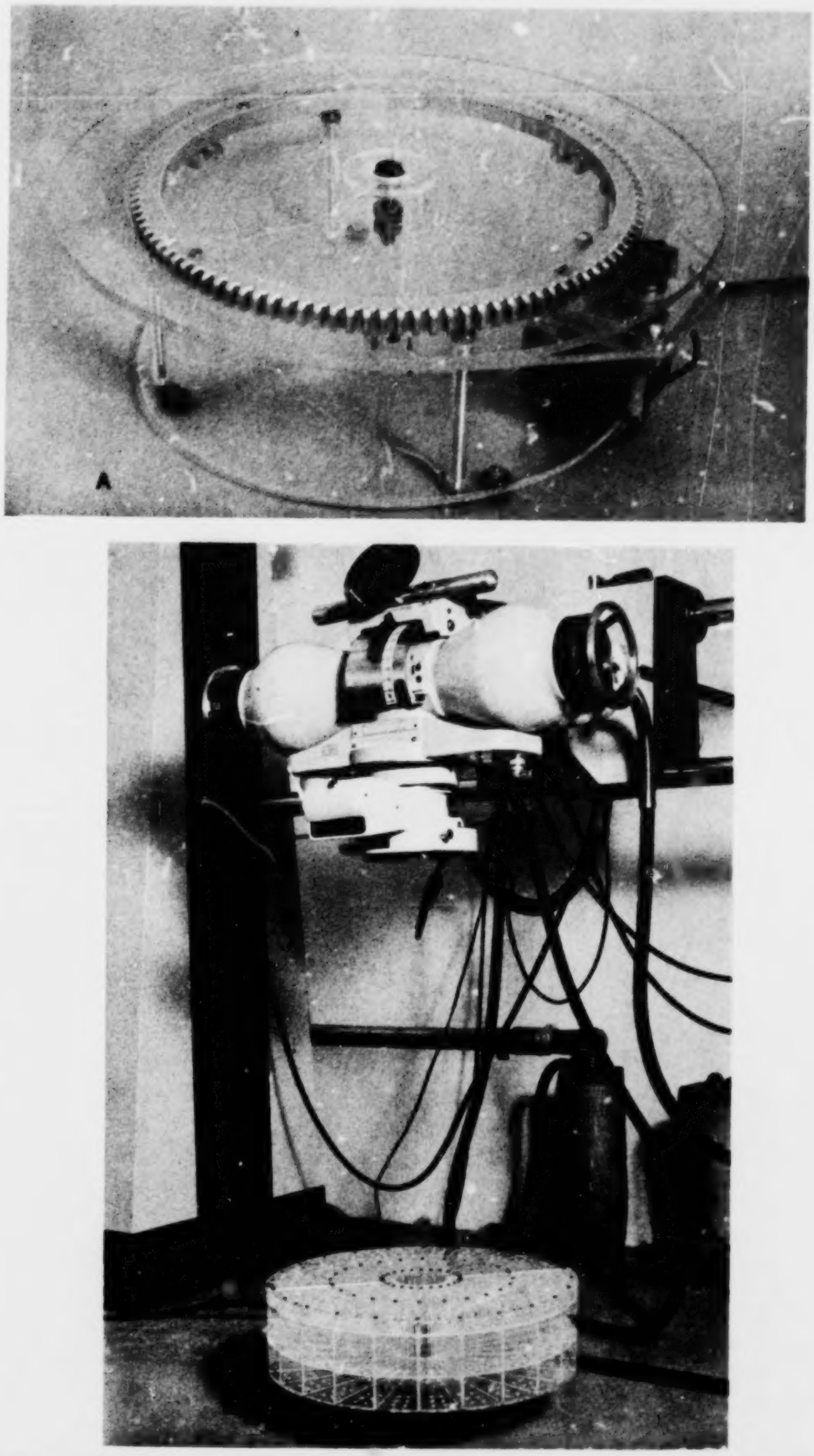


Figure 34. Small-Animal Irradiation Chamber. (A) Motorized component rotates animal-holding chamber during irradiation, (B) chamber in position used for irradiating laboratory rodents.

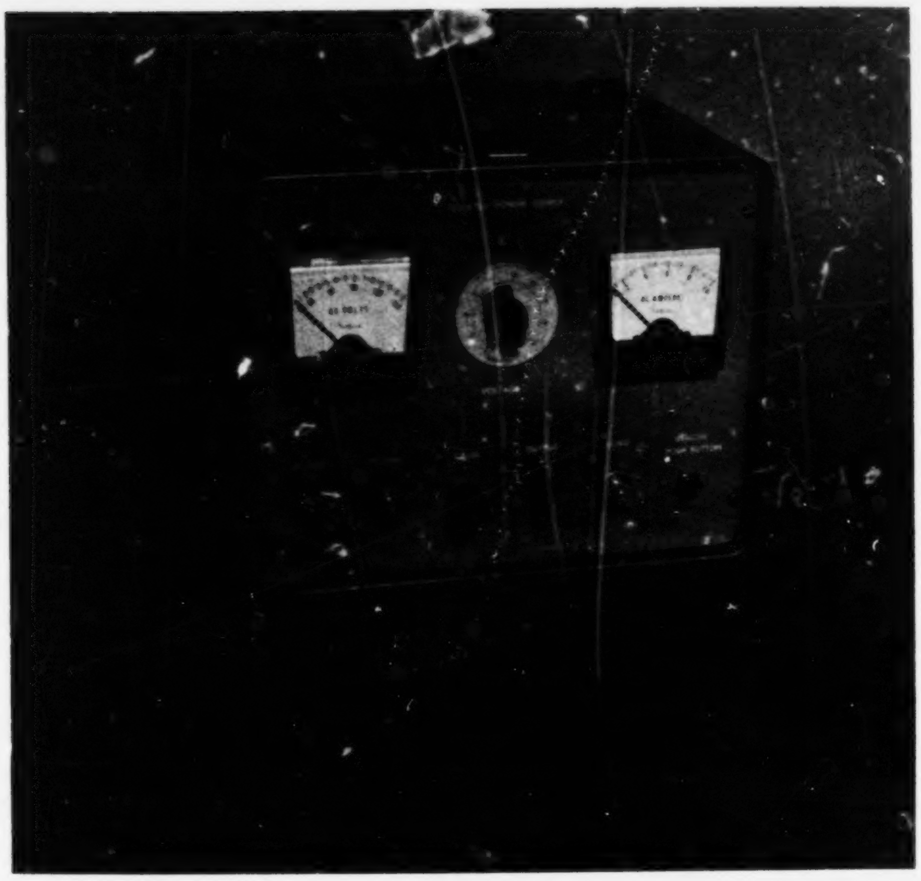


Figure 35. Electrical Device Used to Sacrifice Laboratory Rodents.

The desired characteristics were obtained with a basic transistorized multivibrator oscillator circuit. It was constructed so that a variation in the voltage between two points in the circuit caused a corresponding frequency variation. This dynamic range of oscillation approximates the response of the human ear. If there is no input voltage, there is a very low frequency oscillation which is heard as "motor boating." As the input pulse rate increases, the greater is the transition from lower to higher frequencies and this is audible as an increasingly higher pitched whine. The highest pitch corresponds to the peak of the spectrum being analyzed. A bank of capacitors is included in the circuit so that successive values can be selected to accomodate wide ranges of counting rates. Final details of the capacitor bank are being worked out so that proper time constants can be matched to the various laboratory instruments with which this device will be used (see Figure 36A).

b. Analog-To-Digital Converter

(1) Development of Instrument

The second circuit devised by the Radiation Physics Laboratory--an analog-to-digital converter--pertains to the thermoluminescent dosimeter (TLD) readout system presently employed in the nuclear counting facilities. Development of the circuit stemmed from a desire to investigate and use the glow curves of various thermoluminescent analyzers for further manipulation. We designed this circuit so that the analog photo current signal from the photomultiplier tube in the TLD reader could be converted into a digital pulse that would be acceptable to the analyzer input. Thus, by using this device, glow curves from irradiated and nonirradiated TLD's could be stored in different parts of the analyzer's memory for subsequent operations.

The analog-to-digital converter is shown schematically in Figure 36B. A Texas instrument field effect transistor, normally in conduction, is used in conjunction with a Fairchild integrated circuit voltage-comparator. Small currents (down to 100 pico-amp order of magnitude) are allowed to charge the effective input capacitance of Q_1 and consequently cut the transistor off. The voltage seen at the noninverting input to the comparator begins to approach the comparing reference voltage at the inverting terminal as determined by R_c.

When these two voltages become identical, the comparator yields a positive output condition which in turn fires the tunnel diode D_1 (previously biased just below its transition state). As the diode fires, saturation of the base-emitter junction of Q_3 occurs, allowing it to conduct and apply a negative pulse of time duration determined by the inductor L to the gate of Q_2, a Fairchild MOS field effect transistor. Now as Q_2 conducts, Q_1 is shorted and the process may repeat itself; the repetition rate is governed by the magnitude of the input signal.

Tests indicate that the circuit has a shorting time of approximately 200 nanoseconds and is capable of giving digitized output up to 1 MHz frequency. Finally, to yield a positive output pulse of specified duration (for acceptance by the analyzer) a second comparator was added to the existing circuit. It should be noted that the independent variable of these "glow curves" is "time." Further refinement will permit the use of heating temperature as an alternative variable.

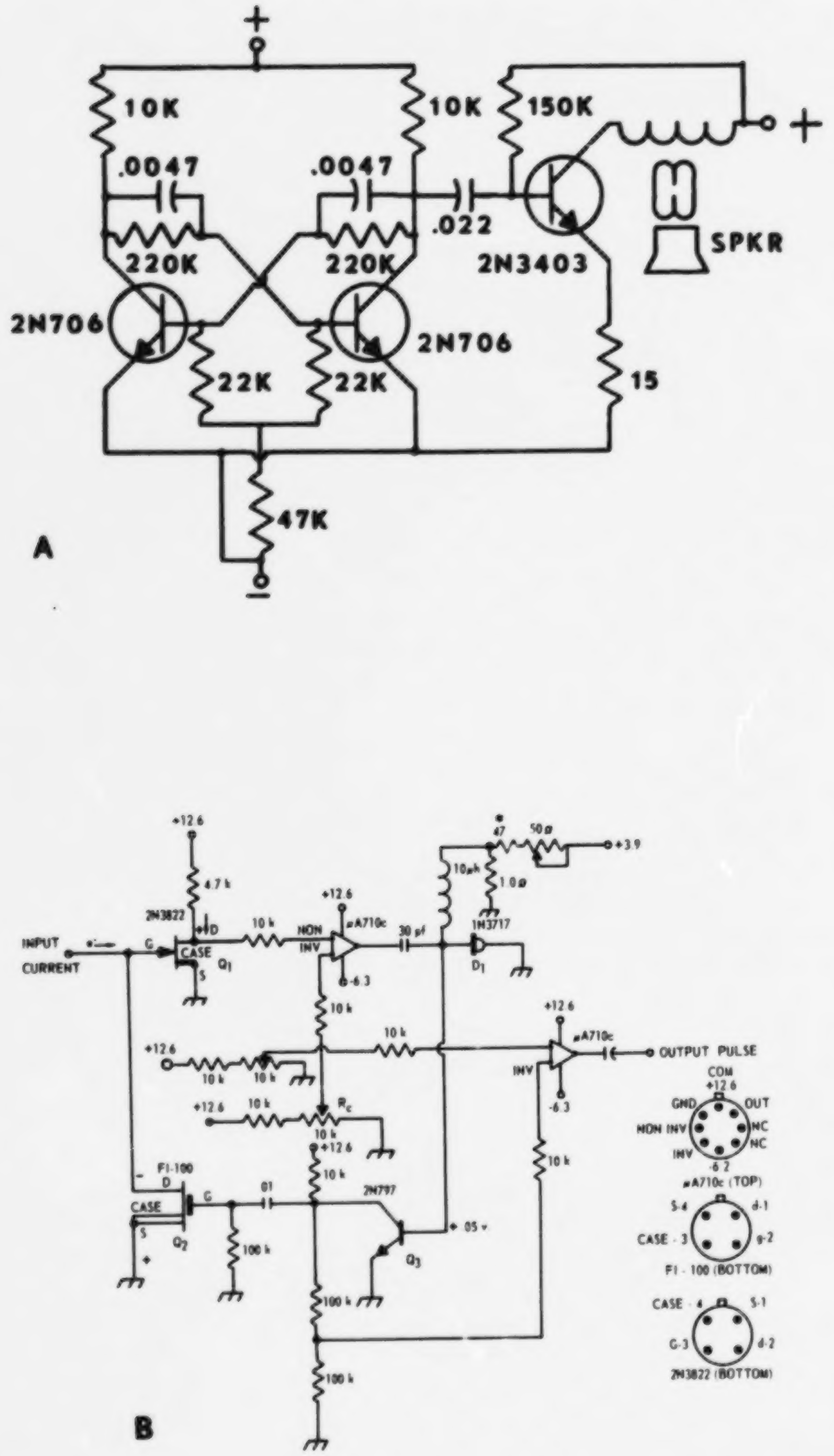

+
10K
10K
150K
+
.0047
.0047
.022
220K
220K
2N3403
SPKR
2N706
2N706
22K
22K
15
47K
−
A
+12.6
4.7 k
2N3822
D
10 k
NON INV
+12.6
μA710c
30 pf
47
50 Ω
+3.9
10 μh
1.0 Ω
1N3717
INPUT CURRENT
G
CASE
Q1
S
-6.3
D1
10 k
+12.6
10 k
μA710c
OUTPUT PULSE
+12.6
10 k
10 k
INV
-6.3
+12.6
10 k
Rc
10 k
+12.6
10 k
COM
+12.6
GND
OUT
NON INV
NC
NC
INV
-6.2
μA710c (TOP)
F1-100
D
CASE
S
Q2
G
01
2N797
+.05 v
Q3
100 k
100 k
100 k
10 k
S-4
d-1
CASE - 3
g-2
F1 - 100 (BOTTOM)
CASE - 4
S-1
G-3
d-2
2N3822 (BOTTOM)
B

Figure 36. Schematic Drawings of (A) Aural Rate Meter, and (B) Analog-To-Digital Converter.

(2) Analytical Relationship Between TLD-System Readout and Photon Spectrum

The glow curve outputs of a TLD system can be stored in the memory of the RCL 256-channel analyzer so that the data for unexposed freshly annealed material and exposed material occupy corresponding halves of the memory. The operational logic modes of the analyzer provide for subtraction of vectors between halves of the memory and integration of a function between arbitrary limits. This technique minimizes the influence of spurious peaks in the glow curves, provides either peak or integral calibration of the TLD system, enables visual inspection of the glow curve for each sample measured, allows greater accuracy in the mR-range of exposures and, last but not least, is fast.

The applications of this technique are many. The principal current interests are phantom studies (tinea capitis), background studies (Kerala, India, for example), cell culture dosimetry, and depth dose data for soft X rays (like those emitted by color TV). Obviously, a wide range of photon energy spectra must be considered in their relationship to the readout of a TLD system. The analytic functions which follow are not complete but should aid in the design and interpretation of experiments of this type.

The total intensity, I (energy/area-time), is related to its distribution in energy, E,

$$I = \int I(E)\, dE = \int \frac{dI}{dE}\, dE = \int E\frac{dN}{dE}\, dE = \int EN(E)\, dE, \qquad (1)$$

where N is the number of photons. The exposure, X, may be expressed in terms of the photon spectrum, N(E), by the inclusion of the mass absorption coefficient of air, $\mu_a(E)$, and an appropriate dimensional constant, ζ,

$$X = \int dX = \zeta \int \mu_a(E) I(E)\, dE. \qquad (2)$$

From the preceding we may also observe that

$$\frac{dX}{dN} = \zeta \mu_a(E) E = X(N). \qquad (3)$$

For every circuit configuration, the TLD system and radiothermoluminescent material have an associated energy-dependent sensitivity, $g_x(E)$, whose units, though arbitrary, are freqeuntly millivolts per Roentgen, mV/R. The total millivolt response of the system, G, is related to the energy distribution of photon flux density:

$$G = \int G(E)\, dE = \int \frac{dG}{dE}\, dE = \int g_x(E)\frac{dX}{dE}\, dE, \text{ or}$$

$$G = \zeta \int g_x(E)\mu_a(E) E\frac{dN}{dE}\, dE \qquad (4)$$

Various single values of the sensitivity, g_i, are frequently measured or derived from experiments conducted with unknown exposure spectra, $X_i(E)$, and an ionization chamber so calibrated to give a correct response, X_i, to a similar spectrum. The sensitivity is

$$g_i = G_i/X_i = \int g_x(E)\frac{dX}{dE}\,dE \Big/ \int \frac{dX}{dE}\,dE = \langle g_x \rangle, \qquad (5)$$

therefore,

$$g_i = \bar{g}$$

That is, the experimentally determined sensitivity is the average sensitivity, an intuitively obvious result.

If we now refer back to Equation 4 and the introductory remarks, we see that the TLD system response, G, depends upon the sensitivity, $g_x(E)$, the photon flux density, $N(E)$, and the limits of integration. These factors should be considered in the planning stage of any application of the TLD system.

B. SPECTRA AND PERCENTAGE DEPTH DOSE OF X RADIATION EMITTED BY A COLOR TELEVISION RECEIVER

H. D. Youmans, G. E. Anderson, W. Lee

Any device capable of accelerating electrons to an energy of several thousand electron volts should be considered to be a potentially dangerous source of X radiation. The color television receiver is such a device and has been shown to produce amounts of X rays in excess of the recommendations of the National Committee on Radiation Protection and Measurements (NCRP),[1], under certain conditions of construction and operation[2]. The emissions have been found to originate in cathode ray, shunt regulator, and high voltage rectifier tubes. Near the cabinet, the beams have cross sectional areas which vary from only a few square centimeters to several hundred. Previous studies[3-7] have delineated this subject with emphasis on radiation protection recommendations and parameters of interest to the manufacturer.

This investigation was conducted to provide an unambiguous determination of the kinds of X rays emitted by a conventional color television receiver and to obtain data which enable the calculation of dose in various organs. The study consists of the characterization of the X-ray emissions from a single color television receiver by means of a multichannel scintillation spectrometer, the matching of the radiations from a comparatively high intensity X-ray machine to those of the television unit in terms of their spectra, and the subsequent use of the X-ray machine to measure percentage depth dose. The depth dose data were obtained in a water phantom with LiF-teflon disc dosimeters and a conventional thermoluminescence dosimetry system.

The color television receiver was a table model with a wooden cabinet. Thin-window G-M counter and ionization chamber survey instruments were used to locate X-ray emissions in the horizontal plane for the shunt regulator (6EF4) and high voltage rectifier (3CN3) tubes. A beam of narrow cross section was found to be emitted from the 6EF4 through the bottom of the set. The spectra of these radiations were measured and are shown in Figure 37.

---

[1] National Committee on Radiation Protection and Measurements (NCRP) Radiology, 75:122 (1960).

[2] Murphy, Emmet, Stewart, H., Coppola, S. and Modine, N., "X-ray emission from shunt regulator tubes for color television receivers," MORP 67-1 National Center for Radiological Health, U.S. Public Health Service.

[3] Braestrup, C. B. and Mooney, R. T. X-ray emission from television sets, Science 130:1071-1074, October 23, 1959.

[4] Callender, M. V. and White, D. F. Aspect of the emission of X-rays from television receivers. J. Brit. I.R.E. 21:287-400, (1961).

[5] Ciuciura, A. "Assessment of X-radiation from television receivers" J. Brit. I.R.E., 19:480 (1959)

[6] Bourne, L. B. X-ray hazards from television apparatus and cathode ray tubes. Lancet, 6941:510 (1959)

[7] Hayashi, S. et al. X-rays from color television receivers, J. Radiation Research, 5-3-4:147-158, (1964).

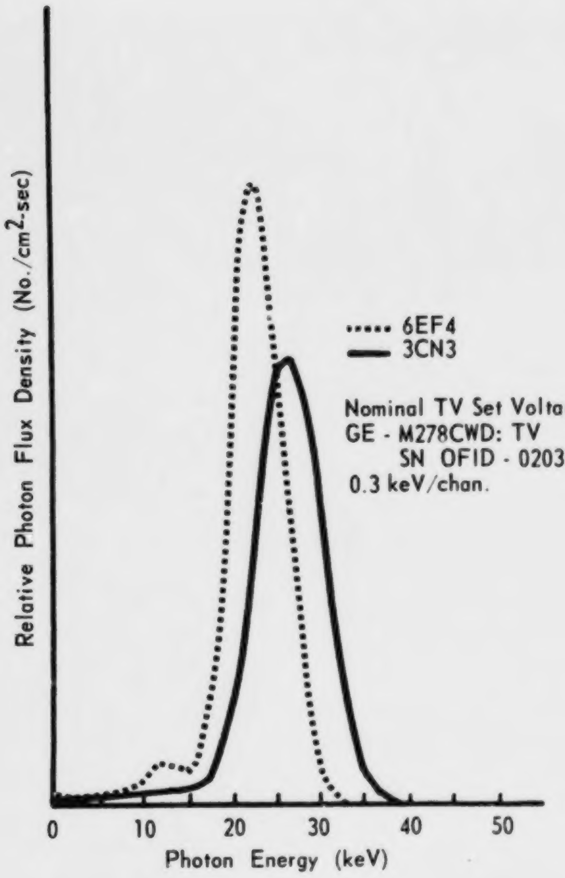


Figure 37. Spectra of X Rays from Shunt Regulator (6EF4) and High Voltage Rectifier (3CN3) Tubes.

Having determined the spectra of the X-ray emissions of the color television receiver, it was then necessary to obtain information which would facilitate the calculation of organ dose rates for arbitrary exposure rates at the skin surface. Knowledge of the central axis depth dose function is most useful for this purpose. Tabulations are available which include depth dose values at low X-ray energies, but the kilovoltage-beam filter combinations represented relate to spectra other than the television receiver emissions.

The direct experimental determination of depth dose data may be readily accomplished with thin discs of radiothermoluminescent material and a water phantom. However, the amount of dosimeter exposure required to yield accurate results necessitates an exposure rate at the surface of the phantom somewhat greater than that available from the TV receiver. This problem was surmounted by utilizing an ordinary superficial therapy X-ray unit (Picker "Zephyr" model NR-2) with the X-ray tube operated at a lower voltage than is usual and the primary beam filtration adjusted to effect a good approximation to the spectra of the TV receiver radiation.

It is apparent that the 33.5 kVp and 24.5 kVdc spectra of Figure 38 are not a perfect match; their average energies differ by 4 keV. Similarly, the 26 kVp and 24.5 kVdc spectra of Figure 39 differ by 3 keV in average energy. A good perspective of this question may be obtained by considering that kilovoltage and first half-value thickness are generally considered to adequately define beam quality in the selection of depth dose tabulations. The first half-value layer of the TV emission is approximately that of a 100 kVp superficial therapy X-ray beam, 1 mm of aluminum. A difference in mean photon energy of 4 keV for similar spectra is measurable but hardly sufficient to change the range of depth dose values significantly. It is our opinion that the X-ray machine spectra are sufficiently well matched to the TV radiations to permit the use of the X-ray unit in the determination of percentage depth dose data which are applicable to television receiver emissions.

Water was used to simulate soft tissue. It was contained in a 16-inch lucite cube; no top was used since the X rays were directed downward upon the surface of the water. Since it was desired to measure X-ray spectra at various depths in the water, corresponding to dosimeter locations, tubes which differed in length were individually mounted over a hole in the bottom of the box through which the X rays emerged to reach the detector. Any perturbation caused by the displacement of water was considered to be negligible.

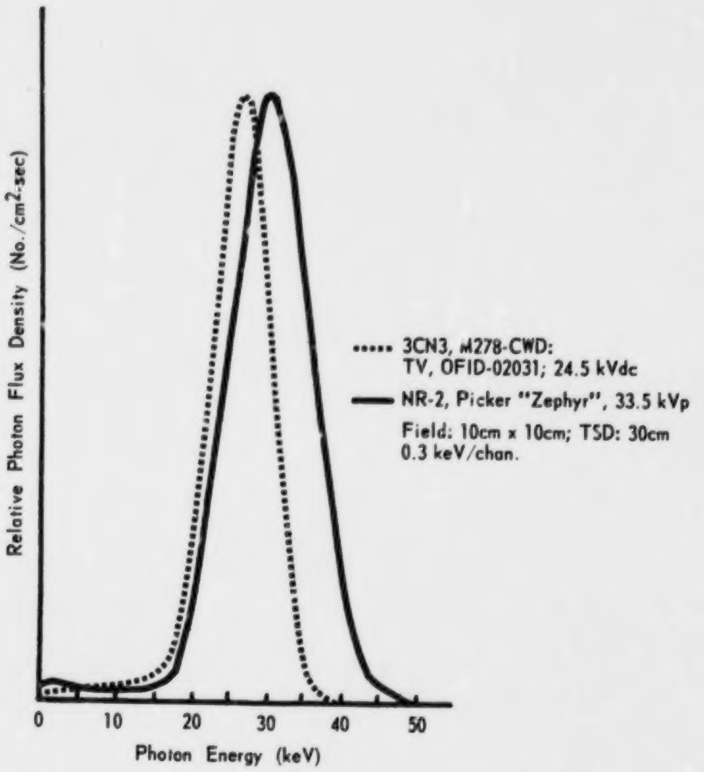


Figure 38. Spectra of X Rays from High Voltage Rectifier Tube (3CN3) and X-Ray Machine (Picker "Zephyr" NR-2).

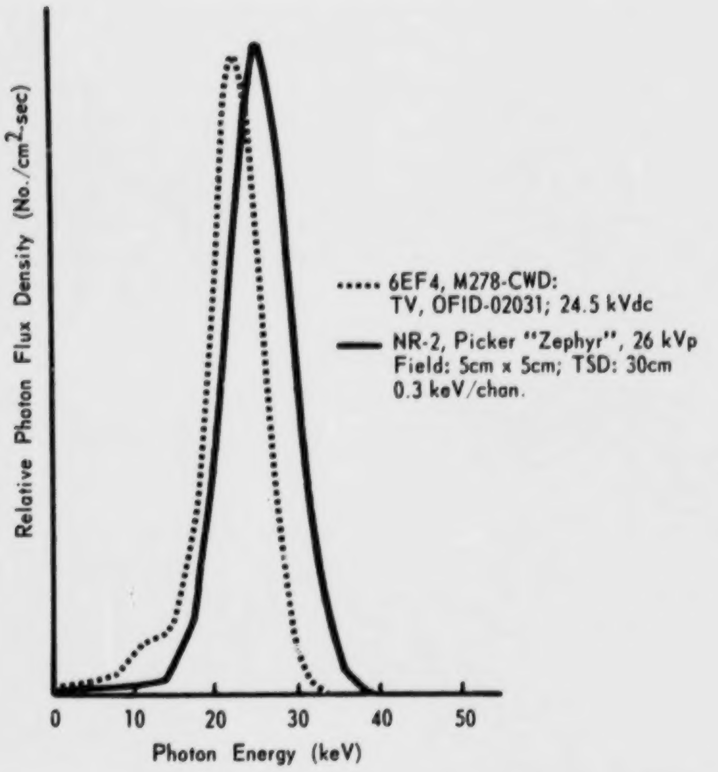


Figure 39. Spectra of X Rays from Shunt Regulator Tube (6EF4) and X-Ray Machine (Picker "Zephyr" NR-2).

The depth dose data were measured with lithium fluoride-teflon disc dosimeters that had previously been calibrated. The dosimeters, held in lucite holders, were positioned at various depths in the water (Figure 40). A commercial thermoluminescence dosimetry system was used to read the dosimeters.

Two depth dose curves were measured at a target-to-surface distance (TSD) of 30 cm (Figure 41). Curve A was obtained with an X-ray tube potential of 33.5 kVp and a beam size of 10 cm x 10 cm at the incident surface. It may be considered to be a limiting case of emission from a high voltage rectifier; the half-value depth $D_{1/2}$ in water is 2.1 cm. Curve B was also taken at 30 cm TSD, but the tube voltage was 26 kVp and the field size was 5 cm x 5 cm. It corresponds more closely to the nominal situation and a shunt regulator tube; the $D_{1/2}$ is 1.55 cm. Comparative spectra, in air, for curves A and B are given in Figures 38 and 39, respectively. Curve C was taken from published depth dose tables[8] and is typical of a 30 kVp medical X-ray beam which has a $D_{1/2}$ of 0.2 cm. It is included for comparison only.

The depth dose data of Figure 41 clearly show that the X radiation from a color television receiver may be appreciably more penetrating

---

[8] Depth Dose Tables for Use in Radiotherapy, Brit. J. Radiol. Suppl. No. 10, (1961).

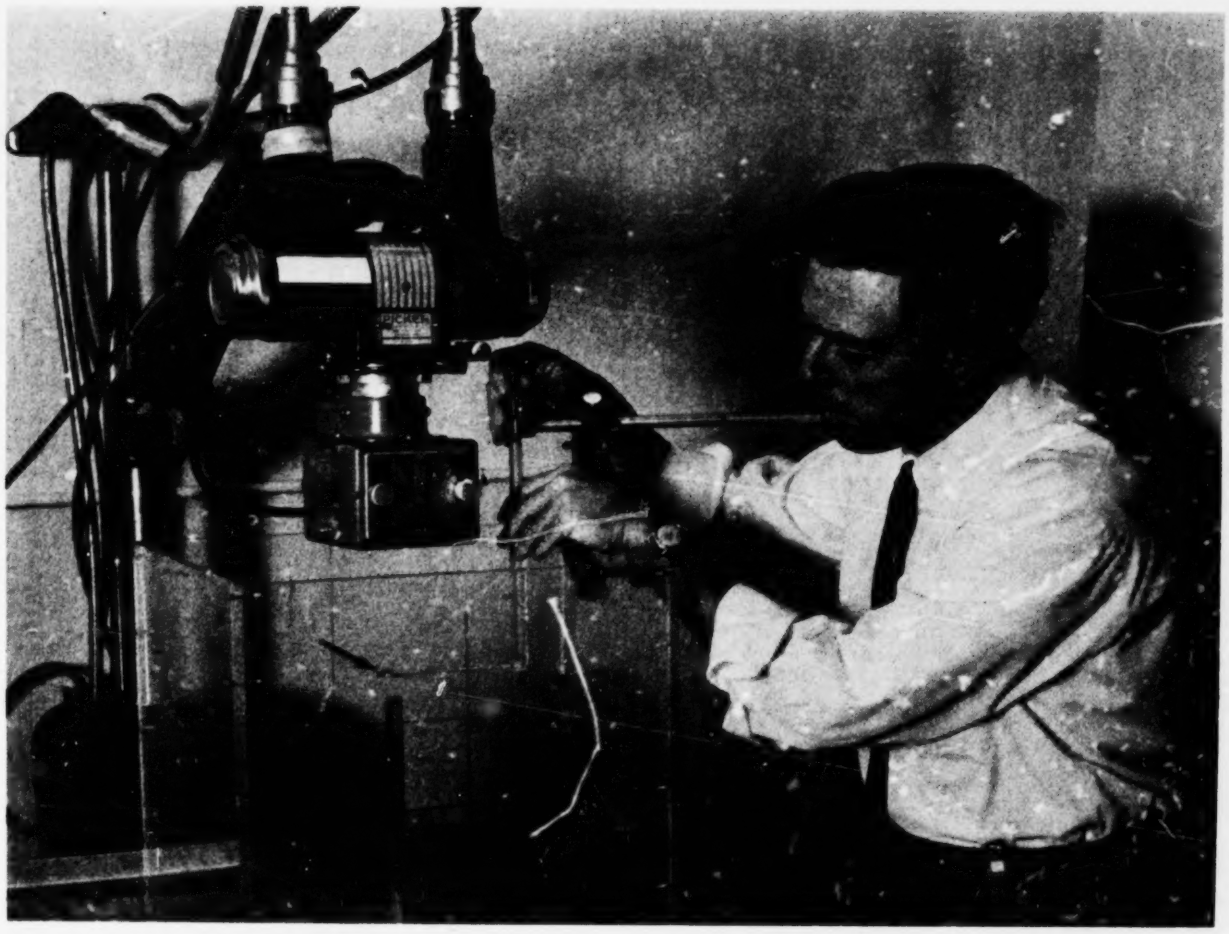


Figure 40. Tissue Equivalent Chamber Used in Depth Dose Studies. The tank is filled with water to simulate tissue. X rays emerge through plastic tubes of different lengths to obtain various depth dose data. Disc dosimeters placed in plastic holder (arrow) could be positioned at required depths.

than a superficial therapy beam generated at the same kilovoltage. In fact, the range of $D_{1/2}$, 1.55 cm to 2.1 cm in water, is typical of medical X rays used in diagnostic radiology or dermatology but which are produced at about 100 kVp. The only medical X-ray beam with similar depth dose properties known is that sometimes used for mammography.[9] It should be appreciated that depth dose is not only a function of the energy spectrum but of field size, field shape, irradiation distance, and tissue thickness and inhomogeneity as well.

---

[9] Egan, R. L. Mammography, p. 74, Charles C. Thomas, Publishers, Springfield (1964).

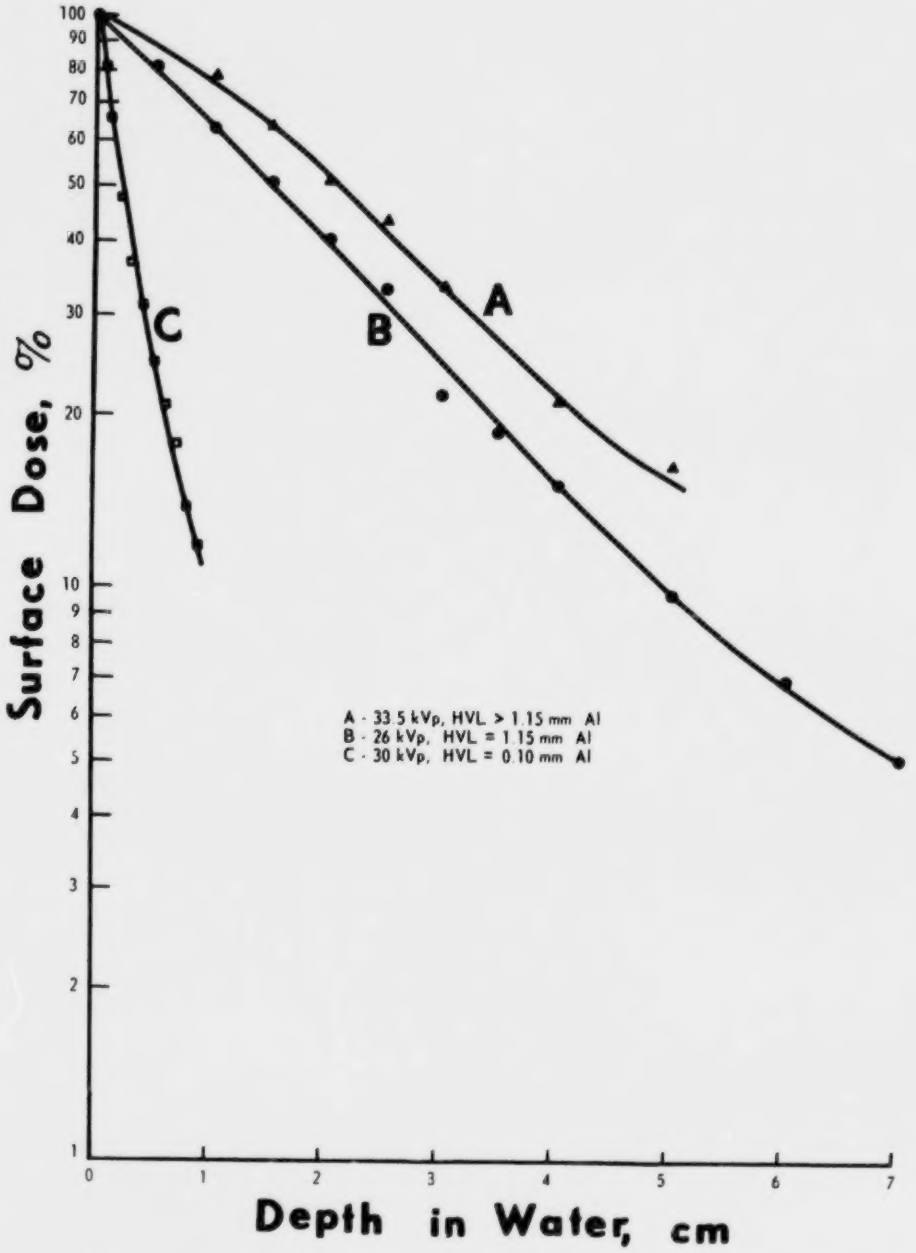


Figure 41. Central Axis Depth Dose Curves.

The data may be applied directly to soft tissue, but special allowances must be made for non-homogeneous media such as bone. Some of these data have been used in the calculation of organ dose rates.[10]

---

[10] Davis, A. K., Youmans, H. D., Wolff, A. H. and Mills, W. A. Considerations on the biological effects of X rays from shunt regulator tubes in color television receivers, Hearings, Committee on Interstate and Foreign Commerce, House of Representatives, 90th Congress, Serial No. 90-11, pp. 97-107 (1967).

C. RADIOLOGICAL ASPECTS OF X-RAY EPILATION IN THE TREATMENT OF TINEA CAPITIS

J. D. Manes, W. Lee, H. D. Youmans

Tinea capitis is a benign fungal infection which has occurred in epidemic proportions among prepubescent children. Prior to the introduction of such drugs as griseofulvin, part of the treatment for the disease required the complete, but usually temporary, epilation of the scalp by superficial X irradiation. Usually a single epilation was sufficient; however, duplicate and in some cases a third X-ray epilation was required. The NCRH is beginning to support an epidemiological study of over 12,000 patients treated between 1948 and 1958 by this method in Israel. X-ray epilation was thought to incur small risk since it was believed that the skull absorbed most of the incident radiation, and that nervous tissue was radioresistant. Recent reports[11,12] indicate that the central nervous system is sensitive to X-ray exposure levels used in the treatment for tinea capitis. Some emphasis has been placed on the cranium, eye and thyroid tissues as well.

The radiological study consists of (i) dosimetric investigations to provide distributions of absorbed dose as well as photon spectra in the skull, brain, eye and thyroid, and (ii) an investigation of morphological and biochemical changes in laboratory animals irradiated in a manner similar to patient treatments.

1. Dosimetric Investigations

W. Lee

At the beginning of the tinea capitis project, our literature survey showed that the absorption of radiation by brain tissue is nearly comparable to that of bone.[13] Based on the experience of diagnostic radiology, this did not seem to be reasonable. Our calculations (Figure 42), based on the chemical composition of tissue from the Biochemist's Handbook and Handbook of Biological Data, show that bone absorbs about five times as much radiation as brain tissue in the energy range of our interest (about 30 keV); the relative mass absorption coefficients of brain tissue and water are nearly identical. Figure 42 also shows that lithium fluoride TLD phosphor is not tissue equivalent. The absorption coefficient is about 20% higher in cross section in most of the photon energy range of interest.

---

[11] Sams, Crawford F. Central nervous system responses to low-level X-irradiation. Prepared for Office of Civil Defense (Contract OCD-05-62-148) through Department of Health, Education, and Welfare, U.S. Public Health Service (December 1965).

[12] Albert, Roy E., et al. Follow-up study of patients treated by X-ray for tinea capitis, Am. J. Public Health 56:2114-2120 (December 1966).

[13] Wachsmann, F. Ausblick auf die Anwendungsmöglichkeiten der Electronenschleuder in der Medizin und bisherige Versuchsergebnisse mit Ultraharten Strahlungen, Acta Radiologica 32:145-158 (1949).

It was initially desired to calibrate the LiF powder, extruded rod, and teflon matrix dosimeters by comparing the responses with Sievert-type miniature ionization (Bg: amber-graphite) chambers. For the following reasons this was impractical. While calibrating the Bg chambers, it was found that they are strongly energy dependent in our range of interest. The response, relative to 115 keV-effective, was 30% high in the 20 to 35 keV range. This is, in part, due to the energy dependence of photon absorption and electron production in the magnesium alloy. Figure 43 demonstrates how absorption by Elektron metal differs from nylon and other common low-Z materials. Also, the precision of measurement was limited by erratic behavior which resulted from microscopic dust from the chamber threads. Dielectric saturation and stress phenomena were apparent but shall not be discussed here. TLD

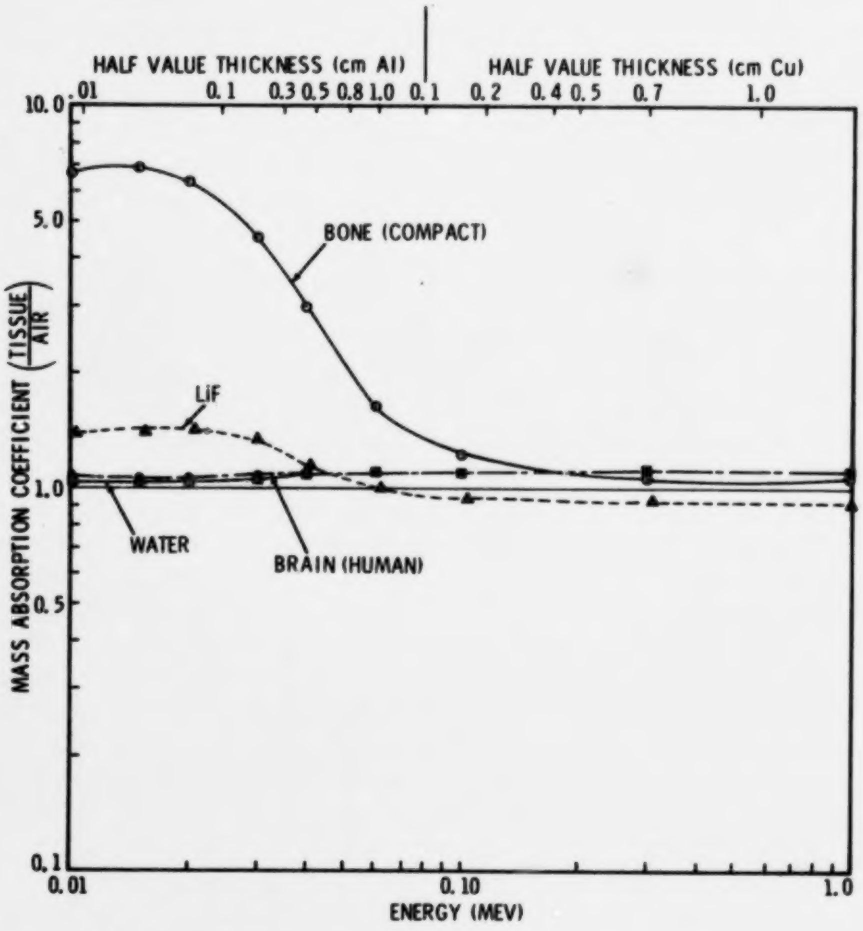


Figure 42. Absorption of Brain, Bone, Water, and LiF at Various Energies.

materials are now calibrated in air relative to the response of an appropriate Victoreen M-570 electrometer and chambers. That is, the absorbed energy in LiF is not considered directly but rather its Roentgen response. Energy-dependent sensitivity of LiF (mV/mg-R) inside the skull phantoms is accounted for by determining the half-value thickness equivalent by a "cap on - cap off" absorber method.

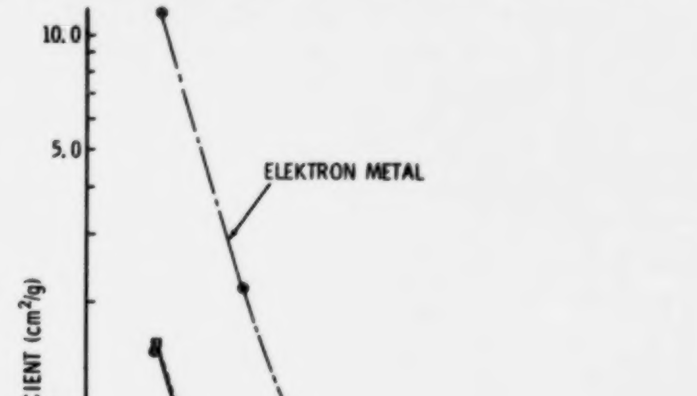


Figure 43. Absorption Coefficient of Elektron Metal.

The average doses in brain, eye and thyroid for skull phantoms of ages 3 and 6 years are presented in Figure 44. The independent variable is X-ray tube potential; it is particularly important because of the strong absorption of X rays by the skull. Three exposure techniques were employed: (1) 63 kVp, 0.66 mm Al HVT; (2) 88 kVp, 1.00 mm Al HVT and (3) 112 kVp, 3.9 mm Al HVT. The source to skin distance was 21.5 cm and the cone was 15 cm in diameter. It should be noted that the eye doses result primarily from scatter internal to the skull; lead eye shields in contact with the eyelids increase the dose by contributing backscatter.

Further studies of this type have begun employing a third skull phantom, 12 years of age, and including a more detailed determination of skull bone marrow and superficial cerebral cortex dose. Gel-microdosimetry may be used where advantageous.

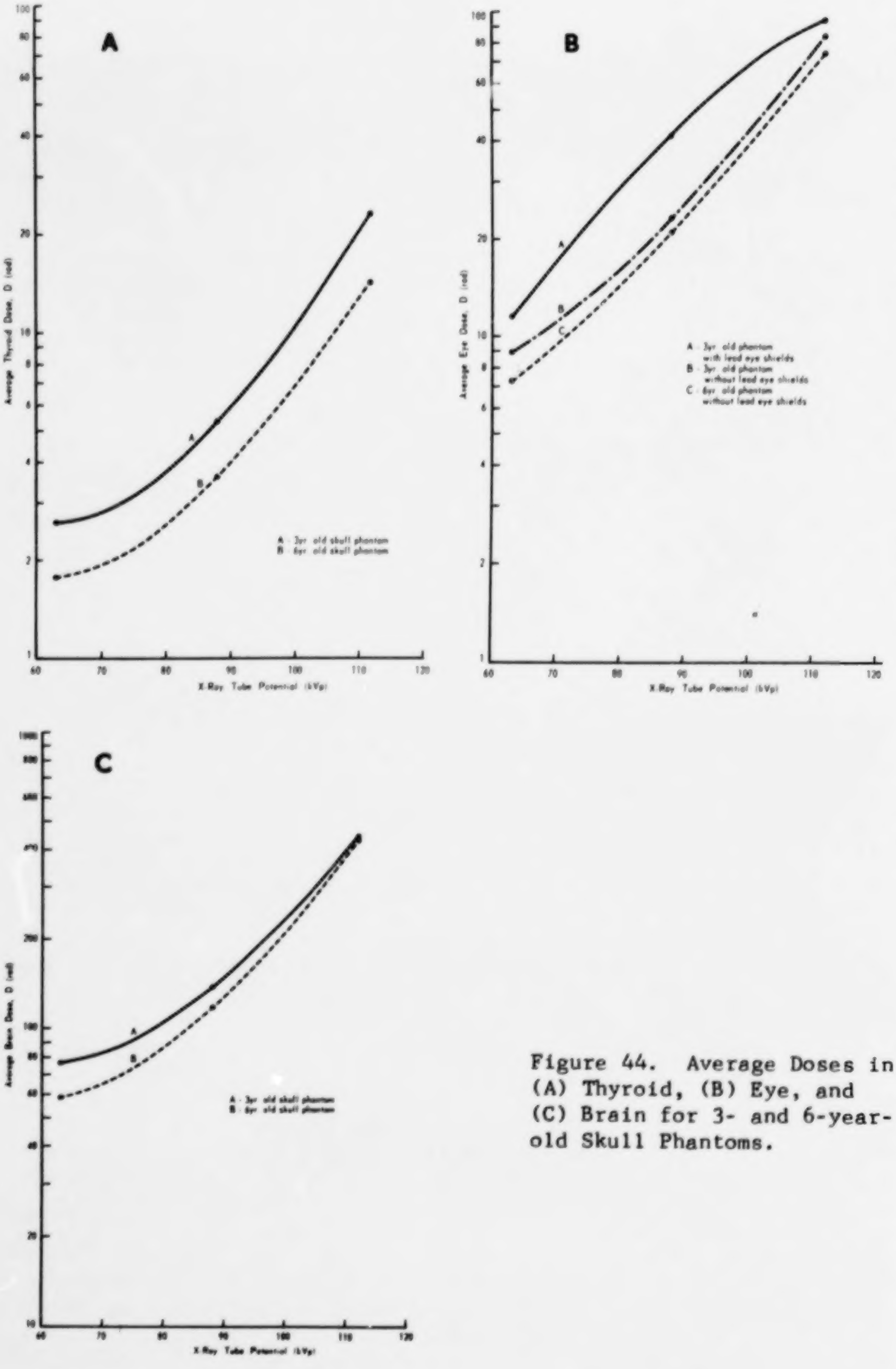


Figure 44. Average Doses in (A) Thyroid, (B) Eye, and (C) Brain for 3- and 6-year-old Skull Phantoms.

2. Morphological and Biochemical Investigations
J. D. Manes

Many investigators have used alterations of structure or functional capacity to assess central nervous system damage by X rays. Recently biochemical changes have been observed in the CNS after exposure to X rays. One such change, demyelinization following exposure to X rays, has been recorded by various investigators.

Most persons treated for tinea capitis were children who were possibly not fully myelinated. Our investigation is designed to evaluate X-ray induced changes in total CNS lipid composition. The study consists of two phases: methods development using adult (11 to 18 months) beagles, exposed to 1000 R and 2000 R, and the basic program with beagle pups exposed to 500 R at 6 weeks of age. Each phase has two groups of dogs, one for morphological studies and the other for lipid analysis. The pups have been irradiated and are to be sacrificed throughout the coming year and the tissues are being prepared for analysis. The discussion and materials which follow relate to the methods phase of the investigation.

The adult animals were anesthetized and restrained when exposed to 100 kVp X rays (half-value thickness of 2.14 mm aluminum) at a source to skin distance of 50 cm (Figure 45). A conventional 6 cm x 8 cm therapy cone was partially blocked with 1/4-inch thick lead to provide a well-defined exposure field of 3 cm x 8 cm. By this means the animals received hemispherical (left cerebrum) irradiation. The exposure rate was 48.8 R/min. (It should be noted that the irradiation of the pups differed in two respects. No anesthesia was used and the entire brain was exposed.) Phantom studies are not yet complete but indicate that the left:right dose ratio in the adult brain is less than 10:1 due to scatter.

The dogs used for the morphological studies were sacrificed by glutaraldehyde perfusion via catheterization of the carotid arteries. The brain was removed and fixed in 4% glutaraldehyde for 1 week. After treating with Cellosolve and pyroxylin the brain was sliced in 6 mm-thick sections and stored in 70% ethanol. Sections corresponding to irradiated and unirradiated sides of the brain were blocked, sliced to a thickness of 6 microns, and stained for microscopic studies with hematoxylin-eosin (universal diagnostic), Luxo-fast Blue - PAS (myelin sheath), and Holmes silver stain (axon and nerve cell bodies).

In most of the brains, we observed changes that are compatible with radiation-induced lesions described by other workers. Such changes include perivascular cuffs of mononuclear cells in many small to medium size vessels in the brain. The cuffs were two to four cell layers wide in some of the sections from animals receiving the higher exposure levels (Figure 46). In these same animals there were often as many as 15-20 cuffs observed in a single section. With lower exposure levels

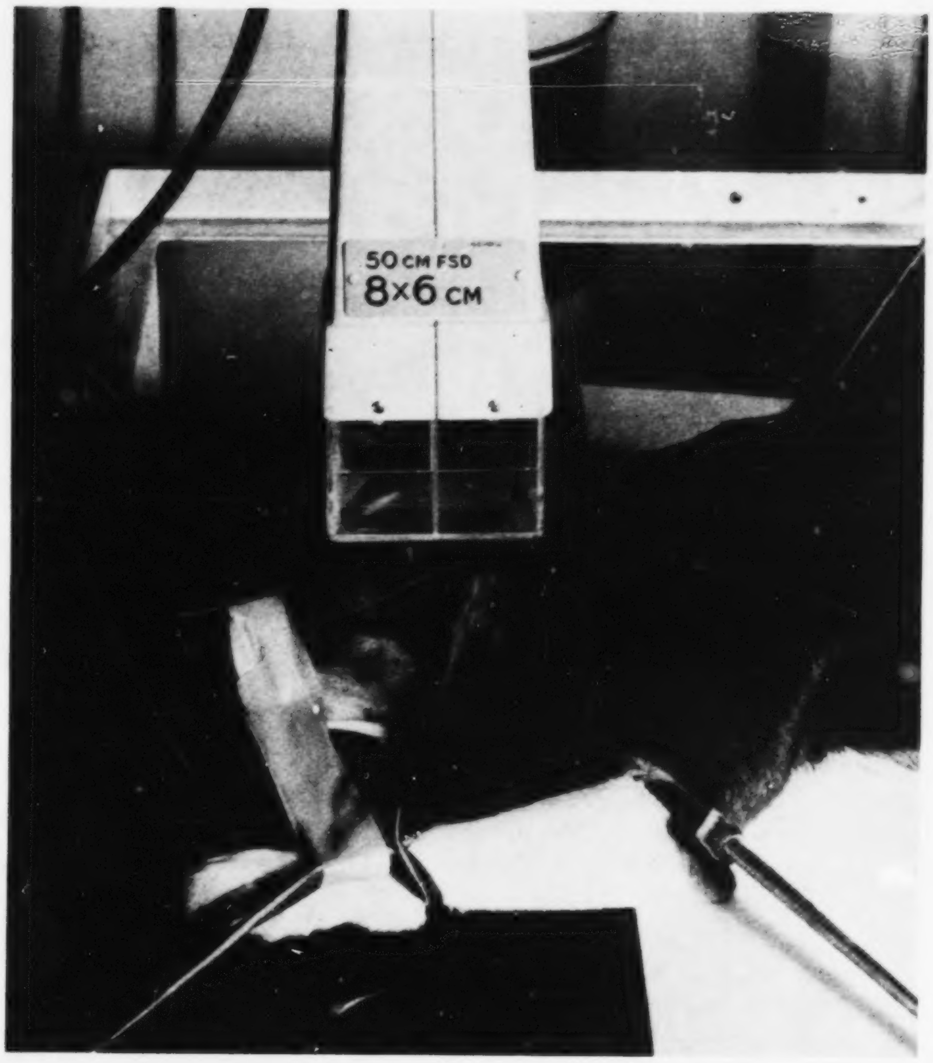


Figure 45. Immobilization of Beagle During X-Ray Exposure of Brain. The left hemisphere was irradiated after the animal was restrained and anesthetized.

and longer postexposure intervals, the cuffs became smaller and fewer in number until at the 1 kR, 120 days post-exposure, no significant changes were found. These lesions may represent a response to primary vascular injury caused by ionizing radiation. Whether the cells composing the cuffs represent in situ replication of vascular adventitial cells or reactive cells from the blood stream cannot be ascertained. It appears that the lesions may be repaired during the 120-day postexposure period. Others have observed this tendency toward repair following X-ray exposure of the central nervous system. The animals should be followed for a longer postexposure time to see if these lesions recur or if the repair is permanent. By modifying the perfusion technique we expect to measure more subtle morphological changes. The animals that are being used for brain lipid analyses were sacrificed by perfusion with two blood volumes of heparinized phosphate

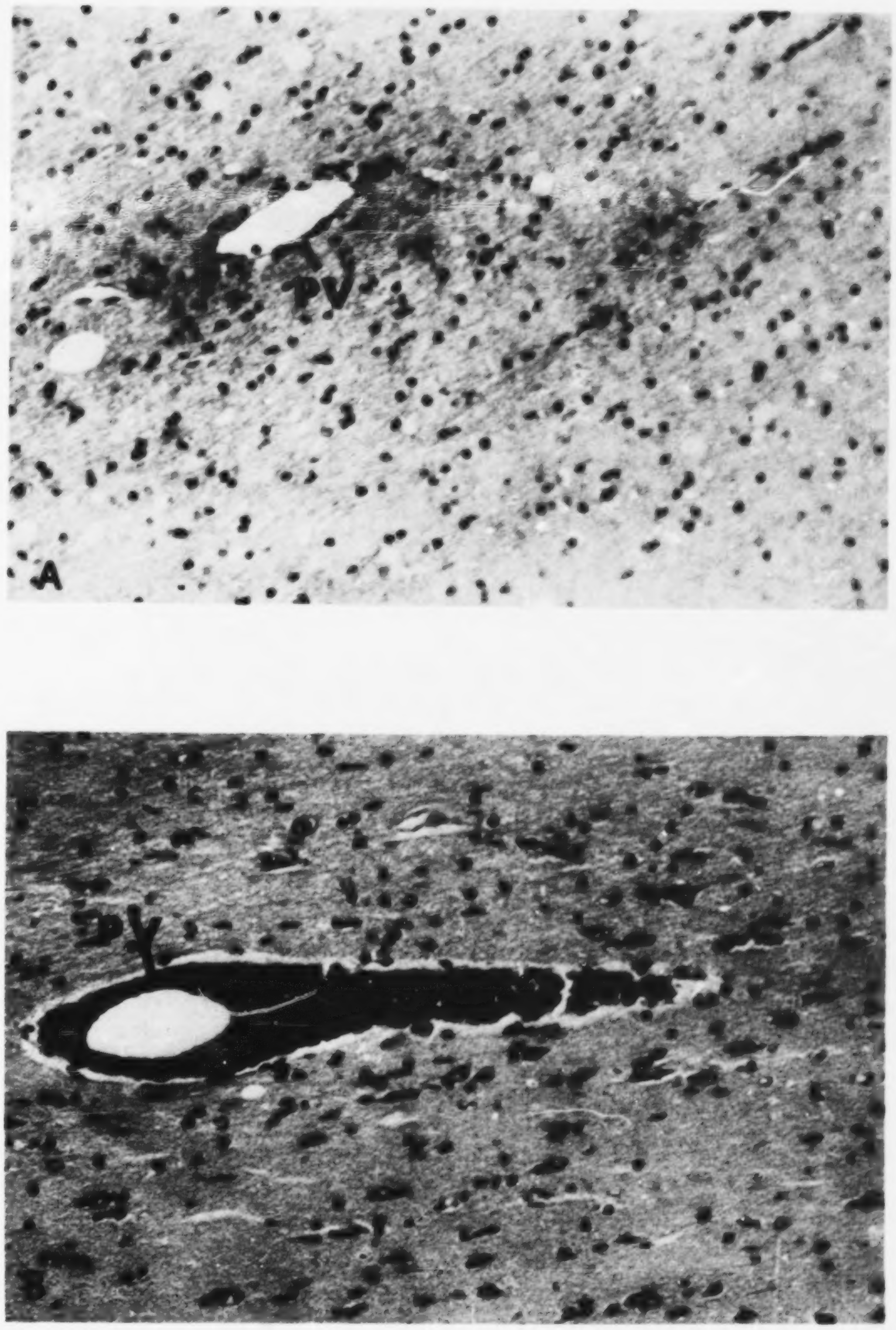


Figure 46. Perivascular Lesion (PV) in the Forebrain. The lesion in (A) was assigned a value of +1 on an arbitrary scale of 4; the lesion in (B) was of the highest order observed (+4).

buffer. The brain was removed within 15 minutes of death and placed in a freezer at -80°F. Approximately 1 [illegible] samples were removed from various areas of the brain for lipid extraction by the method of Folch et al.[14] and analysis by thin layer chromatography. Tissues are currently being assayed; the data have not been analyzed.

Since the animals used in this study were adults, the results cannot be used to infer effects of X-ray exposure of the CNS in children treated for tinea capitis. However, this study did achieve the desired objective, that is, it fixed parameters for future study with young animals. Data are now being collected from animals exposed at 6 weeks of age. These data will enable us to evaluate the risk which accompanies X-ray exposure of the immature mammalian central nervous system in situations comparable to epilation of the human scalp.

3. Additional Lipid Analyses

J. D. Manes

In addition to the lipid analyses connected with the tinea capitis study, our resources were employed to examine the lipid content of several rat mammary neoplasms. These tumors varied in composition from fibrous to glandular. Other investigators have suggested a relationship between large amounts of phospholipid and malignant neoplasms. Furthermore, free cholesterol in large quantities has been reported to characterize rapidly growing tissues. In this study we used thin layer chromatography to characterize and quantitate the lipid components of rat mammary neoplasms after lipid extraction by the method of Folch, et al.[14]

The distribution of 29 tumors by percentage of fat is shown in Figure 47. Table 11 is a tabulation of various lipids occurring in nine rat mammary tumors. The values for free cholesterol are high compared to those reported in the literature; however, the method of analysis, tumor type, and location of the tumor were different. Some workers have used the free cholesterol present in a neoplasm as an index of malignancy. Reports of other workers contradict the reliability of this characterization.[15-18] Our chromatograms revealed that in contrast to other tumor types investigated, the papillary cystadenomas were low in sulfur- and phosphorus-containing lipids.

---

[14] Folch, J., Lees, M. and Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226:497-509 (1957).

[15] Kahn, H. Die chemischen Veränderung bei Krebskranken und ihre Bedeutung für die Serodiagnostik der malignen Geschwülste. Klin. Wochschr. 4:178, 222 (1925).

[16] Bullock, W. E. and Cramer, W. The lipoids of transplantable tumors of the mouse and rat, Proc. Roy. Soc. London, B, 37:236 (1914).

[17] Le May. Lipoides et cancer. Neoplasmes 10:158 (1931).

[18] Bierrich, R., Detzel, A., and Lang, A. Über den Lipoidgehalt bosartiger Geschwültste. Z. Physik. Chem. 201:157 (1931).

From our data it is difficult to correlate lipid composition with a particular tumor type. Free cholesterol values for the tumors examined in this study were high compared to reported quantities in neoplasms. Good characterization and quantitation of tumor lipids were achieved with thin-layer chromatographic analysis.

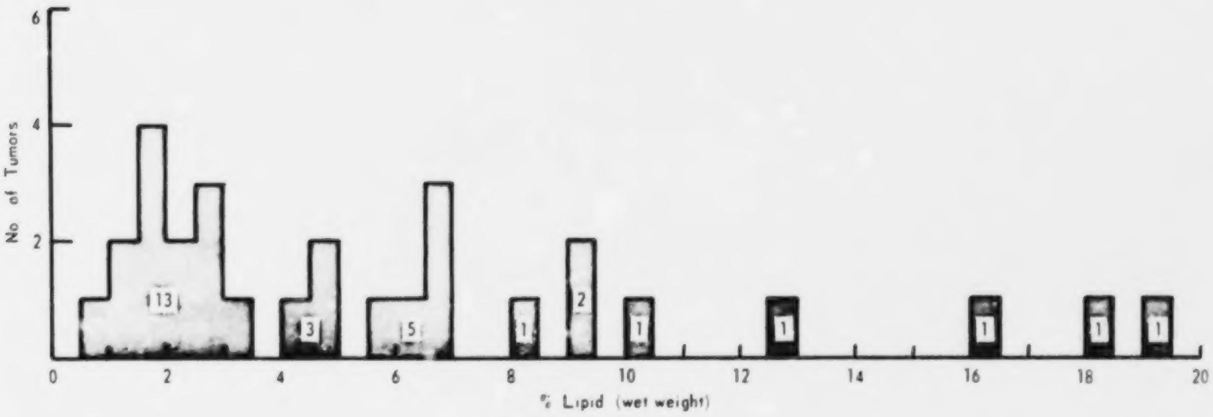


Figure 47. Distribution of Mammary Tumors by Percentage of Fat.

TABLE 11. LIPID COMPONENTS IN RAT MAMMARY TUMORS

Tumor Type[a]	Total Lipid, %	Free Fatty Acids, %	CE/C[b]	CE + C,[b] %	Sulfatides, %	Phosphates, %
FA	6.8	29.5	1.8	42.0	10.3	16.9
FA	1.7	19.5	0.9	45.6	6.8	15.6
FA	2.9	28.0	0.7	38.9	11.8	21.2
FA	2.7	6.1	1.5	50.4	16.8	12.2
FA	4.9	13.9	2.2	43.7	15.4	10.7
F	4.9	4.5	0.6	33.4	19.7	13.9
A	18.2	20.4	1.9	56.7	3.1	19.5
PC	16.0	14.3	-	65.0	4.7	5.9
PC	9.2	19.9	2.5	49.3	4.3	5.9

a. Fa - Fibroadenoma; F - Fibroma; PC - Papillary Cystadenoma.

b. CE - Cholesterol ester; C - Cholesterol.

BLANK PAGE

V. RADIATION CYTOLOGY LABORATORY

W. M. Leach, Chief

Effects of radiation at the cellular level are manifested in a variety of morphologically detectable responses. The cell responses about which research in the Radiation Cytology Laboratory has centered are chromosome breakage, chromosome "stickiness", mitotic delay, and cell reproductive death.

Chromosome breakage may be detected by examining chromosome structural rearrangements. Simple rearrangements, involving a single chromatid or unreplicated chromosome are considered to be consequences of a single ionization per aberration. Complex rearrangements between chromatids, chromosomes, or different regions of the same chromosome, are considered to be consequences of two ionizations per aberration. The possibility that such a conception oversimplifies the origin of aberrations is being emphasized by results of electron microscopic work on chromosome structure.

Chromosome "stickiness" is detected chiefly in cells that undergo division in a comparatively brief period after X-ray exposure. Chromatids of individual chromosomes, as well as chromosomes, adhere one to another in an unspecific non-localized manner.

Irradiation may retard a cell's progress through the cell reproductive cycle and may affect cells in various portions of the cell cycle. This slowing down is generally termed mitotic delay, because fewer cells are dividing after irradiation. Interestingly, radiation delays progress of cells in division in the early post-irradiation period less than it delays progress of cells in other portions of the cell cycle.

What constitutes cell death is problematic. Loss of cell viability is usually equated with failure of the cell to divide. The loss is estimated inferentially by comparing growth of an irradiated cell population with the growth of an unirradiated population.

Some temporal relationships may be made among cellular effects of radiation. Both chromosome "stickiness" and mitotic delay occur mainly in the early post-irradiation period, up to several hours' duration. Both are considered to be transient radiation effects. Chromosomal aberrations are observed most often in mitotic cells of the cell population during and after recovery from radiation-induced delay. Chromosomal aberrations have been shown to be eliminated progressively with successive cell divisions. However, some aberrations may persist for many cell generations.

Our research studies utilize both human and other animal cells for experimental materials. Some studies help to elucidate basic radiation effects on cells; others are aimed toward providing an immediate benefit in human health.

A. HUMAN STUDIES

1. Chromosome Analysis of Patients During Treatment for Chronic Lymphocytic Leukemia

W. Moore, Jr., H. R. Nankin, W. M. Leach

A study of chromosome changes in persons treated for chronic lymphocytic leukemia (CLL) is in progress in collaboration with the Radiation Branch, National Cancer Institute. Patients with CLL who are admitted for therapy at the National Institutes of Health are included in the study. The X-ray therapy varies with the individual, but usually consists of 5 rads daily for 5 days per week, or 12.5 rads twice a week, of fractionated whole-body irradiation. Treatment may continue for 2-6 months. Intermittent rest periods of no irradiation, and follow-up treatments, are arranged as needed. Chromosome analyses are performed before and during treatment, and during remission of CLL.

Blood is obtained from patients at appropriate intervals and the leukocytes are cultured. Culture conditions and techniques, more or less standardized for normal leukocyte culture, were modified in this laboratory to produce satisfactory in vitro growth of the leukemic cells. Chromosomes are examined to determine the number and types of aberrations in the cells.

To date, chromosomes from 12 patients have been analyzed before treatment was initiated. Three patients have remained available for continued study, and chromosome analyses have been performed over a period of at least six months.

Three points stand out on the basis of the limited data available to date. Chromosome damage detected after radiation therapy (e.g., dicentrics, ring formations, or isochromatid breaks) persists for at least one year in peripheral leukocytes of patients in CLL remission. Incidence of aneuploid cells increases with increasing accumulated dose. Finally, incidence of cells with aberrant chromosomes increases with increasing accumulated dose. This point is exemplified in Table 12, which represents the results from one patient after 2 1/2 months of treatment.

TABLE 12. CELLS WITH CHROMOSOME ABERRATIONS IN A HUMAN LEUKEMIA PATIENT

Cumulative Dose (Rads)	Cells Analyzed	Aberrations Number of Cells	Percent
0	373	7	1.9
37.5	161	10	6.2
75.0	151	13	8.6
95.0	100	16	16.0
135.0	110	14	12.7

2. Studies on Probable Etiologies of Congenitally Defective Aneuploid Humans

H. R. Nankin

Some congenitally defective children have either too many or too few chromosomes. It is thought that during gametogenesis or soon after fertilization an abnormality occurs in cell division that causes an unequal distribution of chromosomes to the daughter cells. Accordingly, one cell will have one too many and the other one too few chromosomes. This process is called nondisjunction and may have an etiological role in afflicted individuals.

Research under way in this laboratory is directed at revealing the mechanism of cell division--both normal and abnormal--in short-term cultures of human female lymphocytes. The objective of these endeavors is to find the cause(s) of chromosome nondisjunction which may result in a defective offspring.

Exposure of the pregnant female to X radiation and maternal hyperthyroidism at the time of conception have both been suggested as etiological causes of abnormal children. Present investigations include chromosome studies of hyperthyroid women and in vitro incubation of normal cells with thyroid hormone. Normal human lymphocytes are subjected to X radiation under in vitro conditions. The cells are examined during the stages of mitosis by light and electron microscopy.

3. Electron Microscopy of Chromosomes and Radiation-Induced Chromosome Damage

J. G. Abuelo

Work on the ultrastructure of radiation-induced chromosome damage has been hindered by the lack of knowledge of its normal structure.

Our studies have begun to reveal the ultrastructure of normal human chromosomes. We have observed the longitudinal organization of chromatin fibers along the chromosome arms and the existence of fibrous attachments between dividing chromosome arms. In the course of the ultrastructural investigations, the sequence of the centromere morphology during mitosis has been observed. We are beginning to observe dicentrics, chromatid gaps, and acentric fragments. The electron photomicrograph (Figure 48) illustrates some of our observations. However, a large number of observations will be needed before definite statements about the nature of the radiation damage can be made.

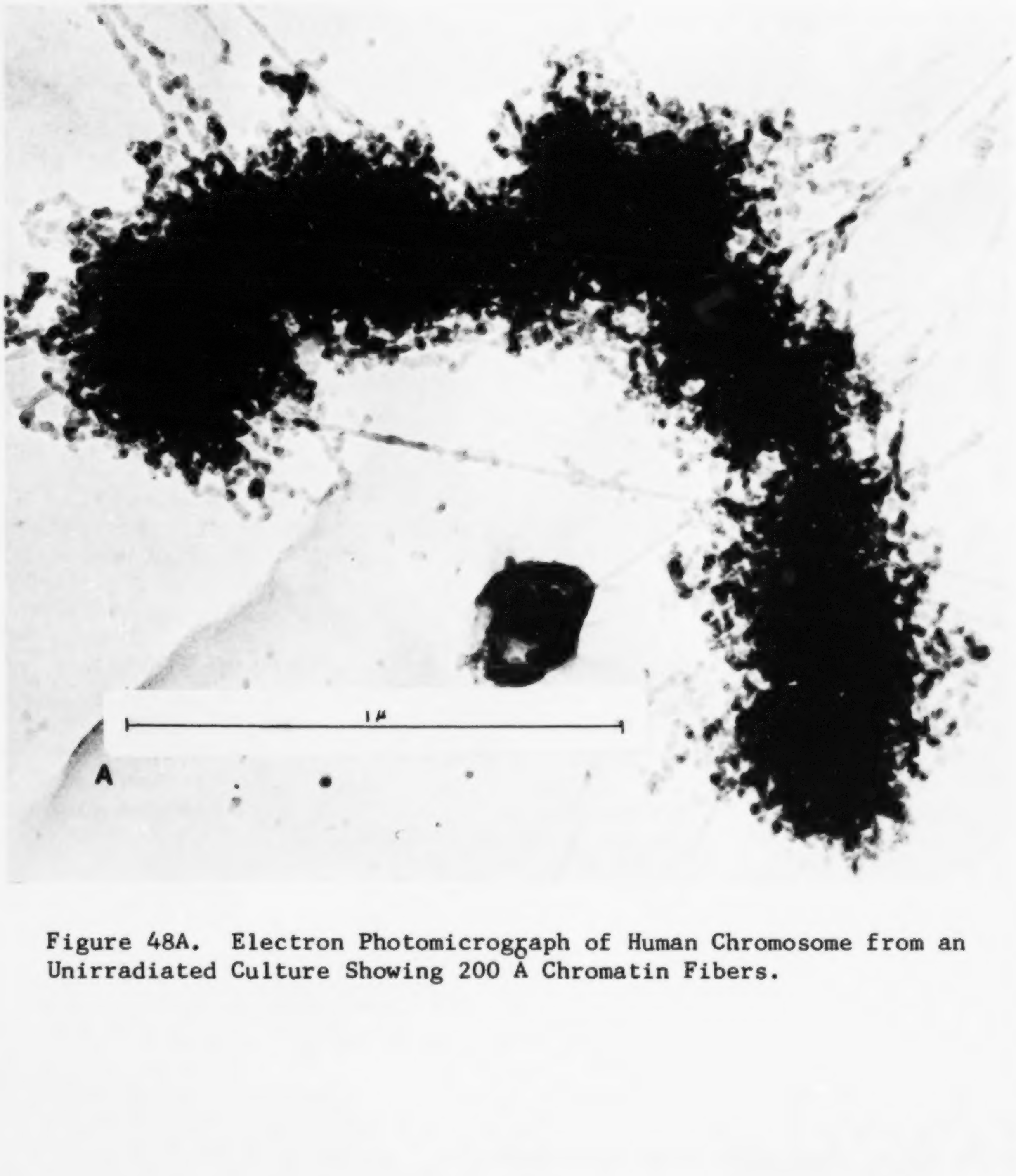


Figure 48A. Electron Photomicrograph of Human Chromosome from an Unirradiated Culture Showing 200 Å Chromatin Fibers.

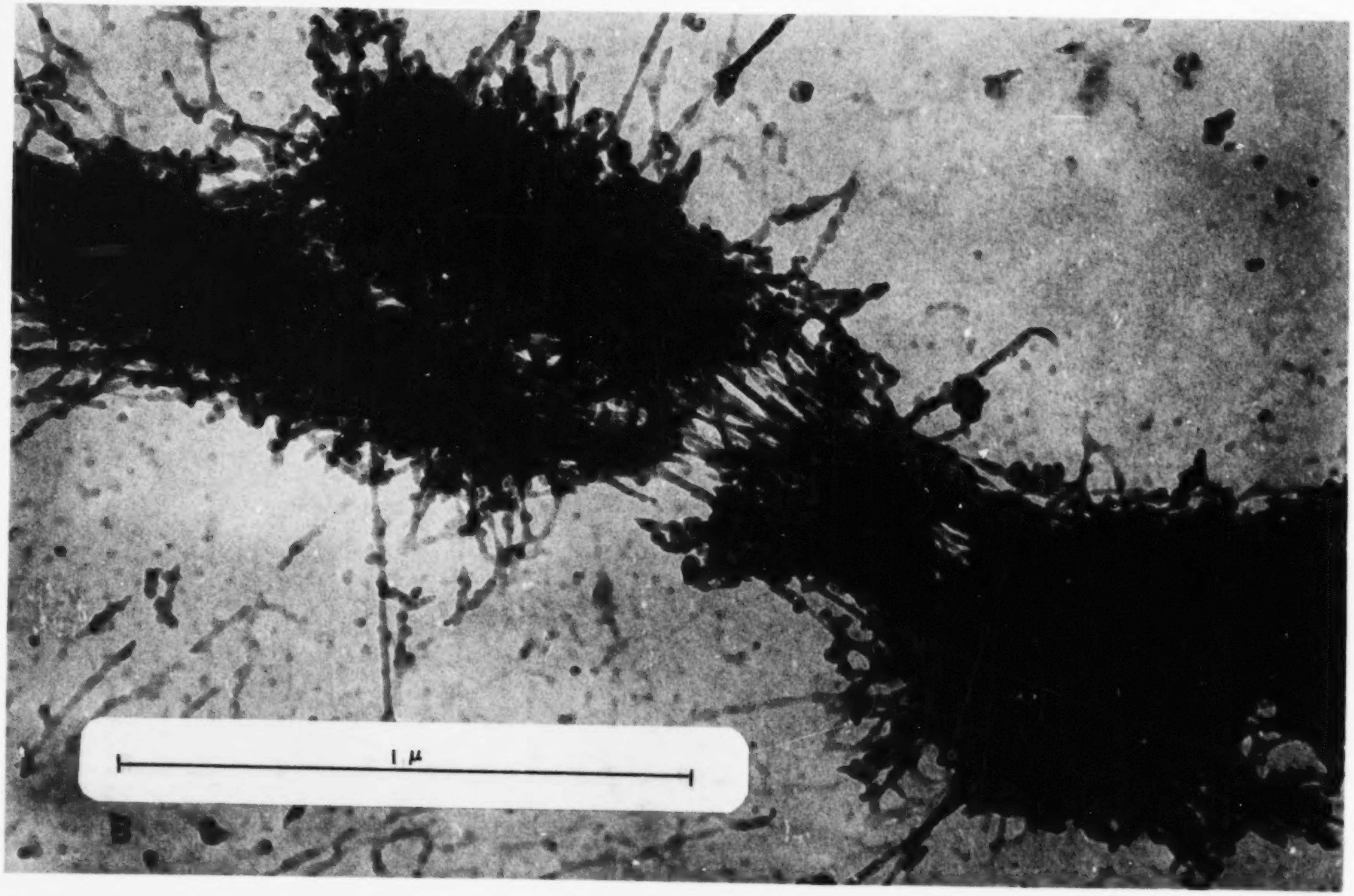


Figure 48B. Electron Photomicrograph of Human Chromosomes
from an Irradiated Culture Showing an Isochromatid Gap.

B. ANIMAL STUDIES

1. Influence of Age on the Incidence of Chromosomal Aberrations in the Thyroid

W. Moore, Jr.

Plausible reasons exist for believing that the thyroid glands of young individuals may be more sensitive to tumor induction by irradiation than the glands of older animals. The difference, if any, may be the result of a difference in thyroid cell sensitivity with age or, more likely, of a combination of factors that affect the metabolism of the thyroid over a period of time. To explore the first hypothesis, the influence of age upon the incidence of chromosomal aberrations in the thyroids of Chinese hamsters irradiated in vivo was studied. The thyroid regions of 7-day-old and 1-year-old animals were irradiated (500 R) and the animals sacrificed 30 days postexposure. The thyroid glands were removed, trypsinized, and the cells grown in culture. The cells were harvested at 72 hours, fixed, and examined for chromosomal aberrations. The percentage of aberrant cells in the thyroids of young Chinese hamsters was 21.9 and in old animals 22.7, suggesting that there is no difference in the incidence of chromosomal aberrations with age. Since cell turnover rates may vary with age, additional work is being done to determine the aberration rate immediately following exposure and with a shortened culture period of only 48 hours.

2. Ultraviolet-Induced Incorporation of Tritiated Thymidine in Grasshopper Neuroblasts During Prophase

W. M. Leach

One of the features of X-ray-induced mitotic delay is the reversion of cells exposed during prophase. Concomitant with the cells' backward movement in the cell cycle, tritiated thymidine is incorporated into acid insoluble deoxyribonuclease digestible chromosomal material. Although this basic observation has been reported in the literature, the effect has not been examined in detail at the cellular level. The purpose of this study was to determine whether the incorporation was the result of the cell movement in the cell cycle or the result of radiation injury.

Ultraviolet radiation was used because reversion of prophase cells is not observed at exposure levels of less than about 5,000-10,000 ergs/mm^2. Neuroblasts of the grasshopper Chortophaga viridifasciata (De Geer) were used for this study because (1) stages of the cell cycle are described in detail and identifiable microscopically in cultures, (2) the duration of prophase is approximately one-third of the duration of the complete cell cycle, and (3) the period of deoxyribonucleic acid synthesis is known in relation to cell stages, and ends in very early prophase.

Neuroblasts in middle, late, and very late prophase were exposed to 256, 512 or 1024 ergs/mm^2 of 2483, 2537, 2650, 2804, or 2967 Å ultraviolet radiation. Incorporation of tritiated thymidine was determined by autoradiography. Each exposed cell stage incorporated tritiated thymidine at each wavelength tested. The incorporation of tritiated thymidine into the chromosomal material apparently resulted from radiation injury and not from reversion in the cell cycle because the energy used was insufficient to cause reversion.

An action spectrum of incorporation was obtained for each cell stage. At the beginning of middle prophase 2537 Å radiation was more effective than other wavelengths in inducing incorporation. The effective wavelength was 2650 Å during early and central portions, and 2804 Å during the terminal portion of middle prophase. A similar shift in the wavelength effective in inducing incorporation was not observed in cells exposed during late-very late prophase.

3. X-Ray Induced Chromosome "Stickiness" in Grasshopper Neuroblasts
W. M. Leach

Hypothetically, the behavior of irradiated deoxyribonucleic acid (DNA) may cause chromosome "stickiness" following X-ray exposure. In a cell population only a small proportion of cells may show "stickiness" effects, yet the viable cells all contain DNA. The cells showing "stickiness" effects contain chromosomes near or at their maximum state of condensation. Part of chromosome condensation may be attributed to coiling of the moiety which maintains the integrity of the chromosome, and part to addition of other macromolecular species to the chromosome.

In a species of grasshopper, Encoptolophus sordidus (Burnmeister), less than 10 percent of embryonic neuroblasts in mitosis contained "sticky" chromosomes after 1,000 R of X rays. In the taxonomically related species, Chortophaga viridifasciata (De Geer) the same exposure produces "stickiness" in about 85 percent of the mitotic neuroblasts.

A qualitative comparison of the cytochemical staining of neuroblasts of the two species was made. The cells were stained differentially for DNA, histone, or ribonucleoprotein (RNP). Preliminary results indicate that DNA and histone staining of the two species is similar, but that RNP is associated more frequently with the chromosomes of mitotic cells of Chortophaga than of Encoptolophus.

4. Survival of Chinese Hamsters Injected with Cultivated Spleen and Bone Marrow Cells After X Ray

K. T. S. Yao

It has been demonstrated that intravenous injection of fresh bone marrow or spleen cells prolongs the survival of mice exposed to X-ray doses in the LD_{50} range. We have conducted preliminary studies of the effects of injecting, intraperitoneally or intravenously, cultivated bone marrow and spleen cells into Chinese hamsters exposed to doses in excess of the LD_{50} (825 R). Both homologous and heterologous cells were used in the study.

The first group of 12 Chinese hamsters was given 800 rads of X ray, and injected intraperitoneally with Chinese hamster spleen cells within 4 hours after the irradiation. One control (irradiated but not injected with cells) died 12 days after X irradiation. One treated hamster (irradiated and injected with cells) died 18 days after X irradiation. All the other animals, including four controls, survived more than 30 days.

The second group of 20 Chinese hamsters was given 1,600 rads of X irradiation and injected with Chinese hamster and rat kangaroo spleen cells. All the control animals that were irradiated but not injected with cells died on the fifth day; all the treated animals died on the sixth and seventh days after X irradiation.

The third group of 12 Chinese hamsters was given 1,200 rads of X irradiation, and injected with rat kangaroo spleen and bone marrow cells. All the controls died in 6 to 8 days, and the treated ones died 9 to 11 days after irradiation. The spleens recovered from the dead animals were prepared for cell cultures. About 50 percent of the cell cultures from the treated animals have been growing well; about 20 percent of the cultures from the control animals have shown cell growth.

The fourth group of 10 Chinese hamsters was given 1,200 rads of X rays and injected intraperitoneally with cultured rat kangaroo heart, kidney, and retina cells. One of the controls died on the fifth day, while the treated animals died on the sixth to ninth days. Another control survived more than 30 days.

5. Radiosensitivity of Chinese Hamster Germinal Cells Cultivated in Rose Chambers

K. T. S. Yao, D. J. Ellingson

The direct observation of radiation effects on germinal cells provides a basis from which the relative degree of radiation effect may be estimated for each of the types of cells in spermatogenesis. Prior attempts by other investigators to establish growing cultures of germinal cells in vitro have not been markedly successful. However, germinal tissues cultivated under a dialysis membrane in Rose chambers in our laboratory continue to differentiate, to retain some normal functions, and to grow for comparatively long periods of time (weeks).

Observations have been directed toward recognizing the different cell types in the seminiferous tubules of Chinese hamsters, and the behavior of the cells during cultivation. Cell differentiation, cell behavior, and cell growth have been serially recorded on films by time lapse photography for systematic analysis.

Growth of Sertoli cells, a sustentacular cell type, is pronounced in all cultures. Migration and division of the Sertoli cells are observed soon after initiation of a culture. The association of Sertoli cells with young spermatids is shown in Figure 49.

Spermatogonia and possibly some spermatocytes contain nuclei which rock back and forth (oscillate), and a number of them even rotate. Nuclear rotation is commonly seen in fresh preparations, and persists for several weeks.

Various modifications of culture techniques have been attempted in order to increase the lifespan of the germinal cells in culture. Meiotic activities of these germinal cells have been positively identified and recorded on film. The transition from spermatid to mature sperm has also been observed and photographed.

Phase contrast microscopic observations and time lapse photography of the living germinal cells have been compared with the appearance in sections and smears of the fixed and stained germinal tissues that were not previously cultured. The comparisons have helped to confirm the differentiation and growth of the cultured cells.

Observations have also been made on germinal cells taken from irradiated Chinese hamsters. Results are insufficient to warrant any conclusions about the relative radiosensitivity of the different cell types of the germinal series.

6. Radiosensitivity of Rat Kangaroo Cell Lines

K. T. S. Yao

We have established eight rat kangaroo cell lines. They were from the tissues of rat kangaroo heart, kidney, retina, bone marrow, spleen, corneal epithelium, corneal endothelium, and bone marrow of a male animal. The heart, kidney and retina cell lines have been growing very well and have been used in our cytogenetic experiments.

The duration of the logarithmic phase of growth of these three lines is 9 days for the heart and kidney cell lines, and 5 days for the retina cell line. The doubling time for heart and kidney cells is 48 hours, and for retina cells 24 hours.

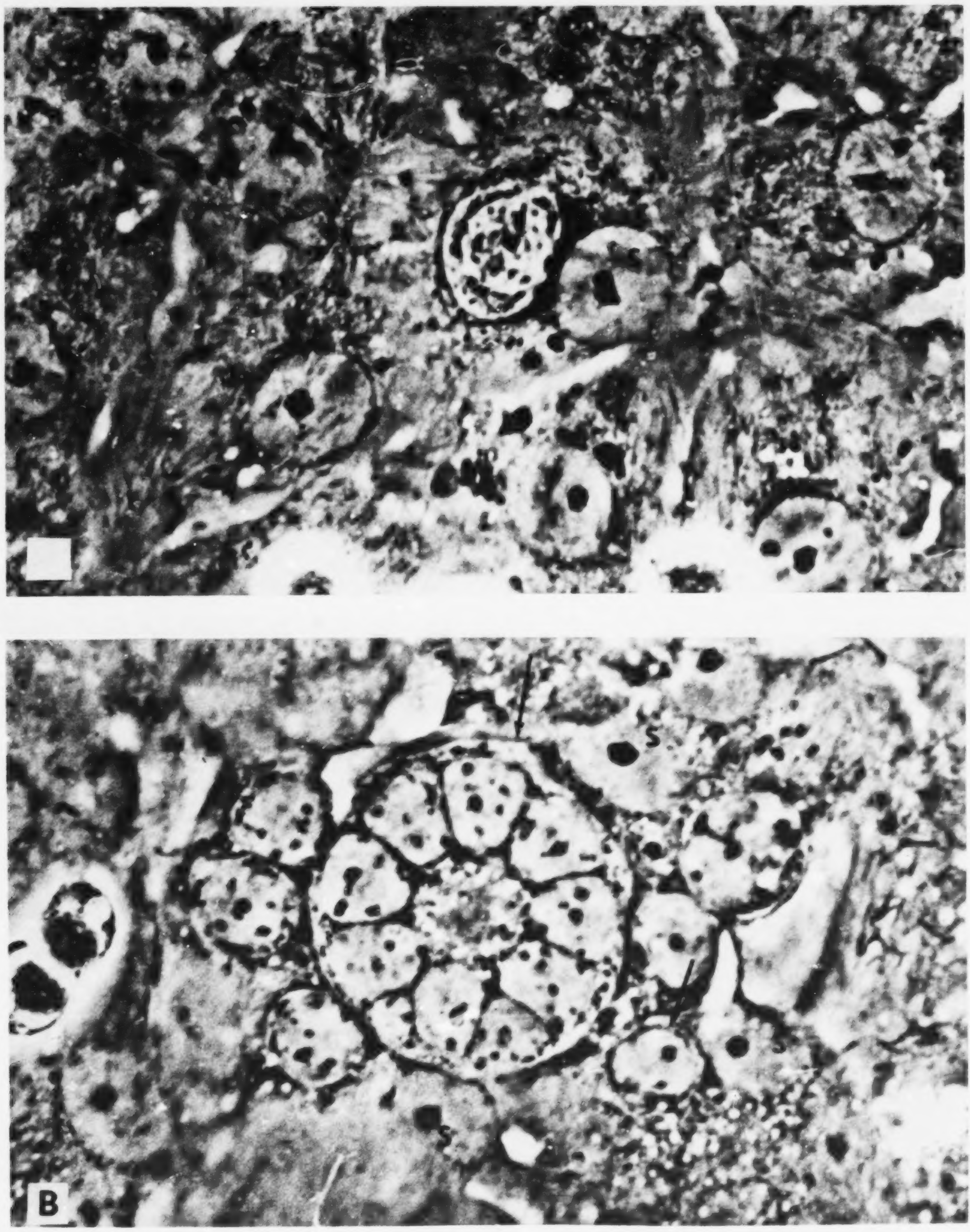


Figure 49. Association Between Sertoli Cells and Germ Line Cells. In A, Sertoli cell (S) associates with a spermatogonium. In B, Sertoli cells (S) associate with a cluster of developing spermatids. The cytoplasm of the Sertoli cells partially surronds (arrows) the spermatids. Magnification about X 1630.

The cells used in the experiments were given 150, 300, 450, 600, 750, 900, 1050 and 1200 rads of X rays. After three days, the cells in each flask were sub-cultured and divided into four flasks. Cell counts were made for each dose of X rays after 3, 5, 7, and 10 days of recovery growth. Of the eight cell lines, postirradiation cell counts are lowest for kidney cells and highest for retinal cells after doses of 150-600 rads. After doses of 400-1200 rads the converse is observed. Cell counts begin to increase from the minimum postirradiation value first in the heart cell line and last in the kidney cell line.

BLANK PAGE

VI. COLLABORATIVE RADIOLOGICAL HEALTH LABORATORY[1]

R. J. Garner, Director

The Collaborative Radiological Health Laboratory was conceived in 1962 as a joint operation between the sponsoring agency, the Radiation Bio-Effects Program of the National Center for Radiological Health and the Department of Radiology and Radiation Biology, Colorado State University. Its purpose is to conduct a long-term study of the effects in the beagle of a single exposure to low levels of radiation with emphasis on investigation of the radiosensitivity of the unborn and young animal primarily in terms of long-term effects. The physical facilities were completed in 1967 with construction of the Radiation Source Building and installation of a 5000 Ci cobalt-60 source in that part of the building accessible from the barrier-maintained area and a 4000 Ci cobalt-60 teletherapy unit in that part outside the barrier (Figure 50).

About three years were spent in developing a cesarian-derived, barrier-maintained (CDBM) colony of beagles to provide the experimental material. The major cost of operation of the laboratory is maintenance of this colony and the supporting programs in clinical pathology and biometry.

A. THE BARRIER-MAINTAINED COLONY

The cesarian-derived, barrier-maintained beagle colony provides the experimental material for this project. It currently comprises approximately 1,700 animals, an increase of about 350 over the course of the year. Much effort is being placed on reducing the size; major culling was not possible until breeding programs appropriate to the needs of long-term work had been established. Criteria have been set which dogs in the long-term project must meet. Culls are being absorbed by short-term studies. The conception rate in the colony has fallen during 1967 probably primarily due to an increase in the number of bitches bred on first estrus. The immunization program has been extended to cover canine hepatitis and leptospirosis.

Since one of the periods of development chosen for inclusion in radiation-effects studies (57 days post-coitus) is approaching term, an analysis of gestation periods has been conducted. The mean gestation period (timed from the day following first breeding) is 61.5 days and

---

[1] Current research conducted at the Collaborative Radiological Health Laboratory is reported in the Annual Report for 1967, Colorado State University - U.S. Public Health Service Collaborative Radiological Health Laboratory, Fort Collins, Colorado (Contract PH 86-62-135).

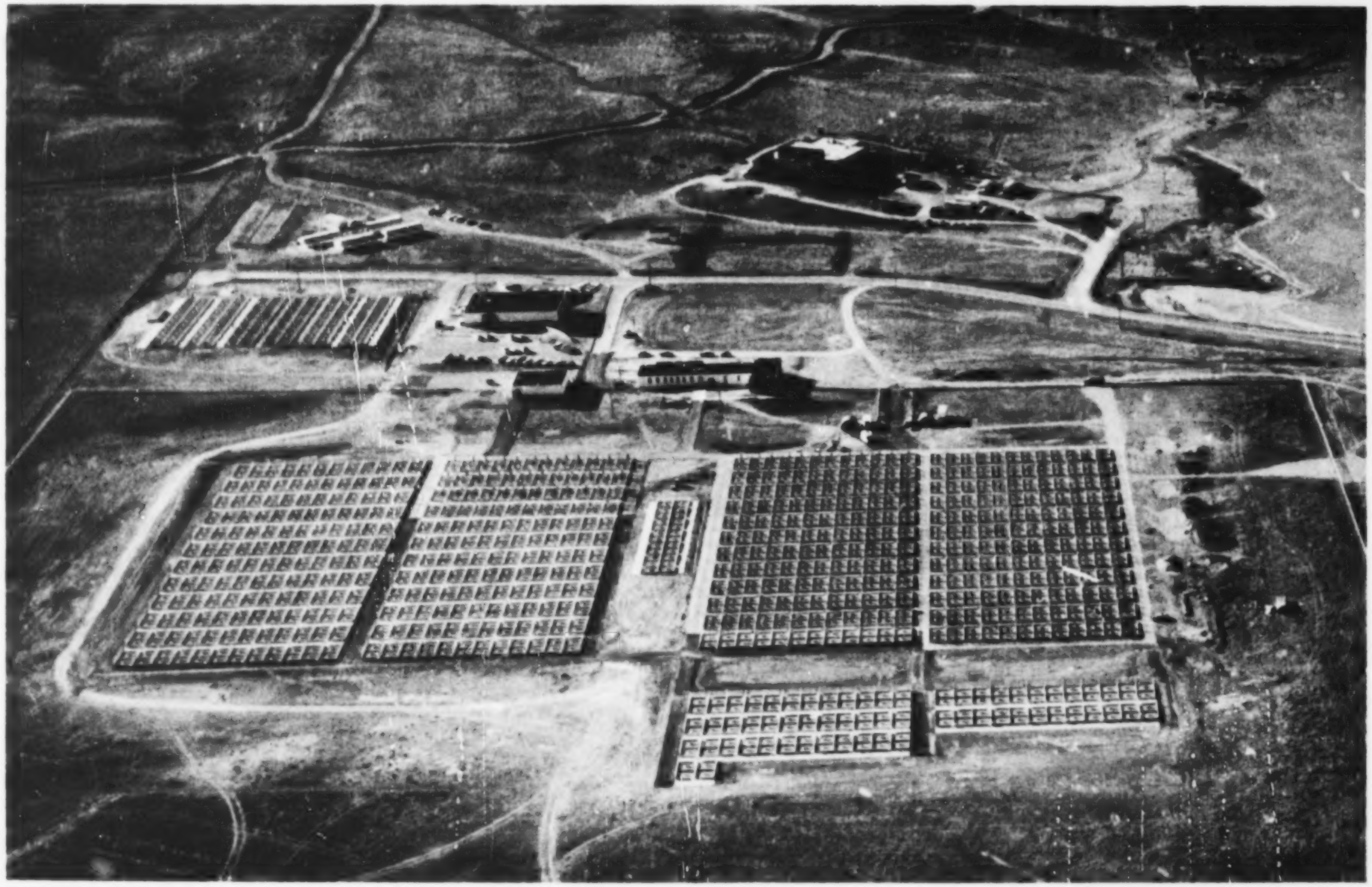


Figure 50. Aerial View of CRHL Beagle Colony, Fort Collins, Colorado. More than 1,700 beagles are housed two to a pen at the Colorado State University Foothills Campus.

the range 55 to 70 days. The approximate 99% confidence limits are 56 to 69 days and, in consequence, it has been considered desirable to change the time of late fetal irradiation to 55 days post-coitus.

The colony has never been completely free from internal parasites; ascarids, coccidia and hookworms are the predominate species. Ova or spirochetes were identified in 6.3% of animals whose feces were examined. An analysis of parasite infestation in terms of season, age, sex, and location of animals within the colony has been made so that the most economical and effective treatment and control measures can be established. It is believed that the greatest benefit can be derived from examination of puppies after weaning.

The current mortality rate in puppies is 22.9%, a figure comparable with other years and other colonies. Deaths prior to weaning account for more than 90% of spontaneous mortality within the colony. Stillbirths (7.2% of the population at risk) and inanition (4.9%) account for the majority of these deaths. Trauma accounted for 2.1% and could be attributed mainly to birth injury. Malformations are of particular interest because of ongoing experiments involving prenatal irradiation; significant malformations were found in 0.8% of the population at risk.

Spontaneous renal and pulmonary calcification has been found in beagle puppies but is apparently a transient spontaneous condition that disappears by about 100 days of age. The condition bears similarities to calcification induced by large doses of vitamin D or parathyroid hormone.

A program was initiated in 1967 to screen the erythrocytes of bitches bled for routine purposes for a possible glucose-6-phosphate dehydrogenase activity. Sex-linked deficiencies of this enzyme have served as useful tools in genetic and cytological studies. About 7% of bitches examined exhibited reduced enzyme activity; the significance of this finding needs clarification.

Serial photographs of all dogs entering the long-term experiment are to be used as part of the records of this experiment. An inexpensive and rapid technique has been developed for this purpose.

B. THE LONG-TERM EXPERIMENT--DESIGN AND FEASIBILITY STUDIES

Research at the Collaborative Radiological Health Laboratory is directed toward examining the long-term effects in the beagle of a single whole-body exposure at different periods of development. Much time has been devoted to setting up protocols which will allow the objectives of this experiment to be achieved efficiently within the restrictions imposed by facilities, staffing, and funding. Six periods of development have been chosen for study: 8, 28, and 55 days post-coitus and 2 days, 10 weeks, and 1 year. The distribution of animals within the experimental design and the choice of exposure levels (0, approximately 20 or approximately 100 R) have been developed to test three hypotheses on the effects of radiation on life-shortening. A total of 1,680 animals will be involved. The first pups were entered into the experiment in December 1967. Commitment of animals will continue over the next 5 to 6 years; the experiment is expected to last for more than 20 years.

Acute lethality studies were necessary as a prerequisite in establishing the long-term design. Currently estimated values for the LD_{50} for cobalt-60 gamma radiation (exposure rate approximately 6 R/min.), using appropriate criteria according to age, are 250, 270, 375, 537, 344, and 416 rads, respectively. The overall pattern of age sensitivity exhibited by the dog thus approximates that reported for the mouse.

The long-term experiment requires the development of sensitive yet atraumatic methods for following changes in structure and function associated with aging and with irradiation. The techniques are being applied to the more immediate problem of defining acute radiation effects, but many of the techniques and procedures will be applied to the long-term studies.

The eye is known to exhibit both immediate and delayed radiation damage. The fundus camera is being explored as a means of recording eye lesions. Cataracts, retinal degeneration, and detached retinas have been found following prenatal or neonatal irradiation. It is suspected that heterochromia iridum may be induced by prenatal irradiation at levels used in diagnostic procedures in man. This condition has been observed to occur spontaneously in the barrier-maintained colony with an incidence of at least 0.1%. Fine structure studies indicate that it is a manifestation of failure of maturation of the chromatophores of the iridal stroma. For investigating visual function it is proposed to use three discrimination tasks: brightness discrimination, visual acuity, and a cognitive-perceptual process (a reversal shift discrimination problem).

CNS function is also being investigated through behavioral studies. These include investigations on discrimination reversal learning, delayed response learning, and avoidance conditioning. The successive discrimination reversal curve obtained with the dog differs from that reported in other species but is similar to the curve obtained (in the dog) for other learning phenomena. The dog apparently finds greater difficulty in discriminating between cues differing in size than in shape, brightness, or vertical versus horizontal patterns. This work has materially aided planning for visual function studies. Exploratory studies resulted in a failure to obtain delayed response in the beagle. Investigation of factors which might account for this failure has led to the conclusion that, because of the strong position habit exhibited by all the subjects, the method adopted is not applicable to investigation of this response in the beagle. In preparation for studies on radiation effects on avoidance conditioning in the beagle, the relative effectiveness of pretraining on this response has been investigated. Pretraining apparently does not increase the rate of avoidance learning.

An organ which is particularly amenable to tests of function under load is the kidney. Attempts are being made to devise methods of determining parameters of renal function using single injection techniques. Preliminary trials have employed external scintillation detectors to measure simultaneously changes in plasma and urine concentration of ^{125}I-Hippuran.

It is almost commonplace knowledge that the hematopoietic system is radiosensitive. In studies on the fine structure of tissues from irradiated dogs, greatly altered leukocytes have occasionally been observed. It is possible that these changes are due to radiation. As an initial step in investigating this possibility, the fine structure of formed elements in the blood of dogs has been examined. The structure of leukocytes, erythrocytes, and platelets is similar to that described for other species; eosinophils differ in that the cytoplasmic granules do not contain the "rod-shaped bodies" seen in man.

C. AGE CHANGES

The interrelationship between the long-term effects of irradiation and the normal aging process is not clear. Hopefully, data from the long-term experiment will throw some light upon this interrelationship. For this reason, parameters were selected for study which are known to show changes with age and which might serve as measures of the biological, as opposed to the chronological, age of irradiated relative to unirradiated dogs.

Growth is an obvious age-dependent parameter and is known to be relatively sensitive to prenatal irradiation. Basic measurements are body weight and skull dimensions. A fourth degree polynomial has been fitted to the body-weight data for initial analysis of these data. Head dimension data have been analyzed using a modified exponential model. Total growth of the skull appears to be most sensitive to irradiation at 10 weeks, less sensitive at 2 days, and effectively insensitive at 1 year. The normal chronology of dental development in beagles in the CDBM colony is being examined; some anomalies in dentition have been encountered. The growth rate of claws and the sinus hairs of the muzzle has been measured using simple methods. Preliminary work suggests that relatively low exposures (100 R) may inhibit hair growth.

Observations of the changes occurring in the hemogram and blood chemistry of the normal beagle from birth through the first two years are recorded.

Cardiovascular function is also under investigation. Both the effects of age and tranquilizers affect the recorded wave forms of the electrocardiogram although tranquilization seems to have no consistent effect. Vector cardiography has been developed until it is now applicable as a semiroutine procedure and has proved useful in following the shift in electrical activity of the heart associated with the change from intra-uterine to extra-uterine life. In an attempt to correlate structure and function, the arterial wall of a 48-day-old puppy and an 8-year-old dog have been analyzed for collagen, elastin, hexosamine, and calcium; pulse-wave velocity and blood pressure recordings have been made in these animals.

Changes in the composition of skin from dogs aged from 35 days gestational age to 11 years have been examined. The hexosamine:collagen ratio, reported to be age-dependent in several species, was found to be significantly age-dependent in the dog over only the first few weeks of postnatal life. However, marked changes in the hydroxyproline content were observed in older animals. It is believed that a combination of hydroxyproline content and water content of skin may prove useful as a measure of biological age in studies on development in the dog.

D. IRRADIATION OF THE EMBRYO, FETUS AND NEONATE

Wherever possible, studies of acute injury following irradiation at various periods of development have been integrated with the preliminary investigations required for designing the long-term work. The policy has been to apply techniques developed for the long-term studies to the more immediate problem of early effects once these techniques have passed the developmental stage. To date, observations have been confined to the effects of irradiation of the embryo, fetus, or neonate.

Two studies are concerned with defining the time and stage of prenatal canine development and characterizing the acute morphological effects of prenatal irradiation. One, still in an early stage, is concerned particularly with the effects of irradiation at 8 or 28 days post-conception--two of the exposure ages chosen for long-term study. The other, initiated in this laboratory and now being pursued in the Department of Physiology and Biophysics, Colorado State University, is concerned with events occurring at the time of implantation. The pattern of response of the beagle embryo to prenatal irradiation has been found to be similar to that described in the mouse with the exception that the conceptus in the beagle appears to be relatively radiosensitive at about the time of implantation.

Work has continued on the qualitative response of the developing retina to exposures in the order of 100 R. Preliminary observations suggest that there is an increased frequency of necrosis of differentiating nuclear layer cells at exposures as low as 10 R. The possibility has been investigated of using the latency of summed visually evoked EEG potentials as a means of evaluating radiation-induced changes in the visual system. In spite of demonstrable morphologic damage in the retina, no significant difference was found in initial peak latency between unirradiated animals and animals that had received 280 R on the 57th day of gestation. There may, however, be a difference in amplitude of response and the acquisition of new equipment will allow this possibility to be explored.

A detailed study of postnatal growth and differentiation in the cerebellar cortex of the dog has been completed and used as the basis for evaluating radiation effects on the cerebellum. In animals irradiated on the second postnatal day, exposures as low as 85 R cobalt-60 gamma radiation produced highly significant changes in the mean widths of cortical layers. Marked qualitative alterations were produced by 325 or 500 R. The value of reflex development as an indicator of functional damage to the CNS is being examined. Some twenty reflexes had received attention in pilot studies. Of these, two, the geotactic reflex and the optokinetic reflex, have been chosen for extensive investigation. The incorporation of carbon 14 administered intraperitoneally as uniformly-labelled glucose into amino acids of the brain of the neonate has been examined following exposure to 610 R on the 57th gestational day. Irradiation produced a reduction, relative to sham-irradiated controls, in entry of glucose into the brain, an increase in incorporation of carbon 14 into lactate and pyruvate, a general decrease in the specific activities of some amino acids, and a reduction in the radioactivity of brain protein. The results indicate that radiation inhibits entry of pyruvate into the citric acid cycle, glycolysis not being materially affected, and also inhibits incorporation of glucose-carbon, particularly into ornithine and glutamine.

Irradiation is known to influence enzyme development associated with functional differentiation. Three hepatic enzymes--α-glycerophosphate dehydrogenase (GPD), glucose-1-phosphate uridyl transferase (GPUT), and glucose-6-phosphatase (GP)--and one enzyme, glucose-6-phosphatase, in the kidney have been shown to exhibit characteristic developmental patterns in the beagle puppy. Following exposure to 250 R cobalt-60 gamma radiation on the 28th gestation day, i.e. following completion of major organogenesis, GPD and GPUT in liver and GP in kidney exhibited a modified pattern, thus confirming observations reported in the rat by other workers. However, it was found that irradiation on the 8th gestational day also affected postnatal changes in the activity of GPUT in liver and GP in kidney, suggesting that irradiation even prior to implantation may induce specific damage to the (unknown) mechanisms involved.

E. DATA PROCESSING

The Biometry Section maintains automated up-to-date information on all dogs in the colony and is responsible for providing assistance with data processing and analysis. Colorado State University has recently acquired a CDC-6400 computer, requiring that previous programs and systems be adapted to the new system. This is currently under way. The generation of pedigree records has been updated. Examples of the types of programs available for data analysis relate to the "F" and "t" statistic, fitting a general linear model and fitting a fourth degree of polynomial. With input from the various systems of the laboratory increasing as more studies develop, the function of the biometry section becomes of even greater importance.

APPENDIX A

PUBLICATIONS JANUARY - DECEMBER 1967

Berman, E., Davis, J., and Stara, J. F. A Dental Chart of the Domestic Cat (Felis catus L.). Laboratory Animal Care 17:511-513, October 1967.

Blanchard, R. L. Relationship Between ^{210}Po and ^{210}Pb in Man and His Environment. (IN) Radioecological Concentration Processes, Proceedings of an International Symposium, Stockholm, 25-29 April, 1966. B. Aberg and F. P. Hungate, eds., Pergamon Press, New York, 1967, pp. 281-296.

Blanchard, R. L. Concentrations of ^{210}Pb and ^{210}Po in Human Soft Tissues. Health Physics 13:625-632, June 1967.

Blanchard, R. L., Cheng, M. H., and Potratz, H. A. Uranium and Thorium Series Disequilibria in Recent and Fossil Marine Molluscan Shells. Journal of Geophysical Research 72:4745-4757, 1967.

Blanchard, R. L. and Kearney, J. W. Natural Radioactivity and ^{137}Cs in Alaskan Caribou and Reindeer Samples. Environmental Science and Technology 1:932-939, November 1967.

Bretthauer, E. W. and Black, S. C. Polonium-210: Removal from Smoke by Resin Filters. Science 156:1375-1376, June 9, 1967.

Burmann, F. J. Influence of Grazing Intensity on Cesium-137 Levels in Milk. Journal of Dairy Science 50:1891-1896, December 1967.

Cisar, C. F. and Jayson, G. Effects of Frequency of Cage Cleaning on Rat Litters Prior to Weaning. Laboratory Animal Care 17:215-217, April 1967.

Engel, R. and Spurrell, F. Neurological and Hematological Changes in the Irradiated Burro. Radiation Research 31:559-560, July 1967.

Fred, R. K., Harris, J. G., Parker, H. G., and Shore, M. L. A Mathematical Model of RES Phagocytic Function. Journal of the Reticuloendothelial Society 4:524-542, December 1967.

Fred, R. K. and Shore, M. L. Application of a Mathematical Model to the Study of RES Phagocytosis in Mice. (IN) The Reticuloendothelial System and Atherosclerosis, N. R. DiLuzio and R. Paoletti, eds., Plenum Press, New York, 1967, pp. 1-17.

Fred, R. K., Zaremba, T. M., and Harris, J. G. The Tissue Distribution of Intravenously Administered ^{14}C-Glucan. Journal of the Reticuloendothelial Society 4:437, November 1967.

Garner, R. J. Collaborative Radiological Health Laboratory, contribution to a special feature: Research Animals -- The Law and the Laboratory. Laboratory Management 5:18-25, October 1967.

Harris, J. Y. Effects of Feeding Resin on Levels of ^{85}Sr and ^{134}Cs in Milk. Journal of Dairy Science 50:992, June 1967.

Harris, J. Y. Sodium Chloride Alteration of Mammary Radioiodine Kinetics. Health Physics 13:931, August 1967.

Hazzard, D. G., Withrow, T. J., and Bruckner, B. H. Verxite Flakes for In Vivo Binding of ^{137}Cs in Cows. Journal of Dairy Science 50: 992, June 1967.

Isaacks, R. E., Hazzard, D. G., Barth, J., Fooks, J. H., and Edmondson, L. F. Nutritional Evaluation of Milk Processed for Removal of Cationic Radionuclides. Chemical Analyses. Journal of Agricultural and Food Chemistry 15:295-299, March/April 1967.

Isaacks, R. E., Hazzard, D. G., Barth, J., Walker, J. P., Fooks, J. H., and Edmondson, L. F. Nutritional Evaluation of Milk Processed for Removal of Cationic Radionuclides. Feeding Studies. Journal of Agricultural and Food Chemistry 15:300-304, March/April 1967.

Johnson, N. M. and Blanchard, R. L. Radiation Dosimetry from the Natural Thermoluminescence of Fossil Shells. American Mineralogist 52: 1297-1309, September-October 1967.

Lambert, P. D., Phemister, R. D., and Tietz, W. J. Visual Evoked Responses of *In Utero* Irradiated Beagles. Radiation Research 31:656-657, July 1967.

Leach, W. M. Lack of X-Ray Induced Chromosome "Stickiness" in Grasshopper Neuroblasts. Radiation Research 31:575-576, July 1967.

Leach, W. M. and Carlson, J. G. Ultraviolet-Induced Incorporation of Tritiated Thymidine into *Chortophaga* Neuroblasts During Prophase. Journal of Cell Biology 35(part 2):77A, 1967.

Maletskos, C. J., Keane, A. T., Telles, N. C., and Evans, R. D. Metabolic Data on Injected and Ingested ^{234}Th in Human Beings. Proceedings of Bioassay Conference, Oak Ridge National Laboratory, Gatlinburg, Tennessee, October 13-14, 1966, pp. 191-207.

Martin, A. Content and Distribution of Stable Strontium and ^{226}Ra in Human Skeletons from Wisconsin Residents. Abstracts, Thirteenth Annual Bioassay and Analytical Chemistry Meeting, Lawrence Radiation Laboratory, Berkeley, California, October 12-13, 1967, p. 14.

Martin, A. A Comparison of Some Bone Ashing Techniques. Health Physics 13:1348-1351, December 1967.

Mayes, Mary G. In Vivo Scanning for ^{131}I in the Rat Thyroid. Abstracts, Thirteenth Annual Bioassay and Analytical Chemistry Meeting, Lawrence Radiation Laboratory, Berkeley, California, October 12-13, 1967, p. 16.

Miller, C. W. and Tietz, W. J. Aortic Ejection Time and Pulse-Wave Velocity Alterations with Age in Dogs. Federation Proceedings 26:663, March/April 1967.

Mishkin, E. P. and Shore, M. L. Inhibition by Actinomycin D of the Induction of Tryptophan Pyrrolase by Hydrocortisone. Biochimica et Biophysica Acta 138:169-174, March 29, 1967.

Mishkin, E. P., Shore, M. L., and Davis, A. K. Differential Response of Hormonal and Substrate Induction of Tryptophan Pyrrolase to X-Irradiation. Federation Proceedings 26:668, March/April 1967.

Moore, W., Jr. Cytogenetics in the Beagle. (IN) The Beagle as an Experimental Animal, Atomic Energy Commission, A. C. Anderson, ed., 1967.

Moore, W., Jr., and Gillespie, L. J. Persistence of Chromosome Damage Following _in utero_ Irradiation of the Dog. American Journal of Veterinary Research 28:890-891, May 1967.

Moore, W., Jr., and Gillespie, L. J. Chromosomes of the Raccoon. Journal of Heredity 58:172, July/August 1967.

Nankin, H. R., Ball, M. F., Sode, J., and Merz, T. Satellite Association and Chromosome Trisomy in Thyrotoxicosis. Clinical Research 15: 269, April 1967.

Schmickel, R. Chromosome Aberrations in Leukocytes Exposed in Vitro to Diagnostic Levels of X Rays. American Journal of Human Genetics 19:1-11, January 1967.

Shively, J. N., Phemister, R. D., and Epling, G. P. Sequence of Changes in the Fine Structure of the Retina of Dogs Irradiated During Retinal Maturation. Proceedings of the Electron Microscopy Society of America, C. J. Arceneaux, ed., 1967, p. 166.

Shively, J. N., Phemister, R. D., and Epling, G. P. Alterations in the Fine Structure of the Mature Retina of Dogs Irradiated as Neonates. Experimental Eye Research 6:278-282, July 1967.

Shore, M. L., Lamanna, A., and Mishkin, E. P. Inhibition of the Hormonal Induction of Tryptophan Pyrrolase by Whole- and Partial-Body Irradiation. Radiation Research 31:641-642, July 1967.

Smith, G. E., Tietz, W. J., and Miller, C. W. Heat Loss and Activity of Puppies During Cold Exposure. Journal of the American Veterinary Medical Association 158:1308, 1967.

Stara, J. F. and Berman, E. Development of an Outdoor Feline Colony for Long Term Studies in Radiobiology. Laboratory Animal Care 17:81-92, February 1967.

Stara, J. F., Nelson, N. S., Della Rosa, R. J., and Bustad, L. K. Comparative Metabolism of Selected Radionuclides in Mammals. (IN) Guidelines to Radiological Health, S. C. Skoryna and D. Waldron-Edward, eds., Montreal, Canada, August 1967, pp. 25-33.

Telles, Norman C. Follow-Up of Thorium Dioxide Patients in the United States. (IN) Distribution, Retention, and Late Effects of Thorium Dioxide, Annals of the New York Academy of Sciences 145, Art. 3:675-676, December 11, 1967.

Tietz, W. J., Benjamin, M. M., and Angleton, G. M. Anemia and Cholesterolemia During Estrus and Pregnancy in the Beagle. American Journal of Physiology 212:693-697, March 1967.

Tietz, W. J. and Seliger, W. G. Temporal Relationships in Early Canine Embryogenesis. Anatomical Record 157:333, 1967.

Tietz, W. J., Smith, G. E., Stuart, B. P., Phemister, R. D., and Garner, R. J. Effects of Irradiation of the Beagle Embryo Prior to Completion of Organogenesis. Radiation Research 31:653-654, July 1967.

APPENDIX B

PRESENTATIONS JANUARY - DECEMBER 1967

Cisar, C. F. Safe Handling of Animals Exposed to Radiation and Radioisotopes. American Association Laboratory Animal Sciences, Washington, D. C., October 3, 1967.

Engel, R. E. Necropsy Findings in Desert Bighorn Sheep. Wildlife Society, California-Nevada Section, Sacramento, California, January 27-28, 1967.

Engel, R. E. and Spurrell, F. A. Neurological and Hematological Changes in the Irradiated Burro. Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.

Fred, R. K., Zaremba, T. M., and Harris, J. G. The Tissue Distribution of Intravenously Administered ^{14}C-Glucan. Reticuloendothelial Society, Winston-Salem, North Carolina, December 3-6, 1967.

Harris, J. Y. Sodium Chloride Alteration of Mammary Radioiodine Kinetics. Health Physics Society, Washington, D. C., June 18-22, 1967.

Harris, J. Y. Effect of Feeding Resin on Levels of ^{85}Sr and ^{134}Cs in Milk. American Dairy Science Association, Ithaca, New York, June 25-28, 1967.

Harris, J. Y., Carr, S. A., and Solomon, G. C. Comparative Mammary Histology. Joint Meeting of the Clinical Society and Commissioned Officers Association, Atlanta, Georgia, May 9-12, 1967.

Hazzard, D. G., Withrow, T. J., and Bruckner, B. H. Verxite Flakes for In Vivo Binding of ^{137}Cs in Cows. American Dairy Science Association, Ithaca, New York, June 25-28, 1967.

Lambert, P. D., Phemister, R. D., and Tietz, W. J. Visual Evoked Responses of in utero Irradiated Beagles. Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.

Leach, W. M. Lack of X-Ray Induced Chromosome "Stickiness" in Grasshopper Neuroblasts. Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.

Leach, W. M., and Carlson, J. G. Ultraviolet-Induced Incorporation of Tritiated Thymidine into Chortophaga Neuroblasts During Prophase. American Society for Cell Biology, Denver, Colorado, November 13-15, 1967.

Maletskos, C. J., Keane, A. T., Telles, N. C., and Evans, R. D. Retention and Intestinal Absorption of ^{224}Ra and ^{234}Th and Some Dosimetric Considerations of ^{224}Ra in Human Beings. Symposium on the Late Effects of Internal Radiation, Sun Valley, Idaho, September 12-14, 1967.

Martin, A. Content and Distribution of Stable Strontium and ^{226}Ra in Human Skeletons from Wisconsin. AEC Thirteenth Annual Bioassay and Analytical Chemistry Meeting, Berkeley, California, October 12-13, 1967.

Mayes, M. G. In Vivo Scanning for ^{131}I in the Rat Thyroid. AEC Thirteenth Annual Bioassay and Analytical Chemistry Meeting. Berkeley, California, October 12-13, 1967.

Miller, C. W. and Tietz, W. J. Aortic Ejection Time and Pulse-Wave Velocity Alterations with Age in Dogs. Federation of American Societies for Experimental Biology, Chicago, Illinois, April 16-21, 1967.

Mishkin, E. P., Shore, M. L., and Davis, A. K. Differential Response of Hormonal and Substrate Induction of Tryptophan Pyrrolase to X-Irradiation. Federation of American Societies for Experimental Biology, Chicago, Illinois, April 16-21, 1967.

Moore, W., Jr. and Colvin, M. Persistence of Chromosomal Aberrations in Chinese Hamster Thyroid Following Administration of ^{131}I. Society of Nuclear Medicine, Seattle, Washington, June 20-23, 1967.

Nankin, H., Ball, M., Sode, J., and Merz, T. Satellite Association and Chromosome Trisomy in Thyrotoxicosis. American Federation for Clinical Research, Atlantic City, New Jersey, April 30-May 1, 1967.

Shively, J. N. Fine Structure Changes after Exposure to Irradiation. Mountain States Society of Electron Microscopists, Fort Collins, Colorado, March 15, 1967.

Shively, J. N., Phemister, R. D., and Epling, G. P. Sequence of Changes in the Fine Structure of the Retina of Dogs Irradiated During Retinal Maturation. Electron Microscopy Society of America, Chicago, Illinois, August 29-September 1, 1967.

Shore, M. L., Lamanna, A., and Mishkin, E. P. Inhibition of the Hormonal Induction of Tryptophan Pyrrolase by Whole- and Partial-Body X-Irradiation. Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.

Shore, M. L., Mishkin, E. P., and Lamanna, A. Interference, by X-Irradiation, with Enzyme Induction in the Liver of the Rat. Armed Forces Radiobiology Research Institute Symposium, Bethesda, Maryland, June 8-9, 1967.

Smith, G. E., Tietz, W. J., and Miller, C. W. Heat Loss and Activity of Puppies During Cold Exposure. American Veterinary Medical Association, Dallas, Texas, August, 1967.

Stara, J. F., Nelson, N. S., Della Rosa, R. J., and Bustad, L. K. Comparative Metabolism of Selected Radionuclides in Mammals. Guidelines to Radiological Health, an International Conference, Montreal, Canada, August 17-21, 1967.

Stara, J. F. and Waldron-Edward, D. Repressive Action of Sodium Alginate on Absorption of Radioactive Strontium and Calcium in Cats. Symposium on Diagnosis and Treatment of Deposited Radionuclides, Richland, Washington, May 15-17, 1967.

Telles, N. C. Radiation: Useful and Useless. Glenwood Lions Club, Glenwood, Maryland, October 16, 1967.

Telles, N. C. and Wolff, A. H. Radiation Carcinogenesis - A Review of Factors in Developing Criteria for Radiological Control. American Public Health Association, Miami Beach, Florida, October 22-27, 1967.

Tietz, W. J. and Seliger, W. G. Temporal Relationships in Early Canine Embryogenesis. American Association of Anatomists, Kansas City, Missouri, April 4-7, 1967.

Tietz, W. J., Smith, G. E., Stuart, B. P., Phemister, R. D., and Garner, R. J. Effects of Irradiation of the Beagle Embryo Prior to Completion of Organogenesis. Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.

Wolfangel, R. G., Shaw, S. N., and Christian, J. Investigation of Whole Body Measurements of ^{131}I-Tetracycline as an Index of Skeletal Growth. American Association for Advancement of Science, New York, N. Y., December 1967.

APPENDIX C
ADVISORY COMMITTEES

RADIATION BIO-EFFECTS AND EPIDEMIOLOGY ADVISORY COMMITTEE

Dr. Victor P. Bond, Chairman
Chairman, Medical Department
Brookhaven National Laboratory

Dr. Charles E. Dunlap
Professor and Head
Department of Pathology
Tulane University School of Medicine

Dr. Alan M. Gittelsohn
Department of Statistics
School of Hygiene and Public Health
Johns Hopkins University

Dr. Louis H. Hempelmann
Professor of Experimental Radiology
School of Medicine
University of Rochester

Dr. Edward B. Lewis
Professor of Biology
California Institute of Technology

Mr. Leonidas D. Marinelli
Director, Radiological Physics Division
Department of Radiological Physics
Argonne National Laboratory

Dr. Karl Z. Morgan
Director, Health Physics Division
Oak Ridge National Laboratory

Dr. Richard R. Overman
Professor of Clinical Physiology
College of Medicine
University of Tennessee

Dr. Leonard M. Schuman
Professor of Public Health
School of Public Health
University of Minnesota

Dr. M. Thomas Wagner, Executive Secretary
Assistant to the Chief
Radiation Bio-Effects Program
National Center for Radiological Health

COLLABORATIVE RADIOLOGICAL HEALTH LABORATORY ADVISORY COMMITTEE

Dr. Edward Alpen
Head, Biology and Medical Sciences Division
U. S. Naval Radiation Defense Laboratory

Dr. Allen C. Anderson
Radiopathologist
University of California
School of Veterinary Medicine

Professor Ernest Furchtgott
Department of Psychology
University of Tennessee

Dr. Marvin A. Kastenbaum
Statistician
Oak Ridge National Laboratory

George A. Sacher, Jr., Chairman
Senior Biologist
Division of Biological and Medical Research
Argonne National Laboratory

Dr. Carl F. Tessmer
Chief, Experimental Pathology Section
M. D. Anderson Hospital and Tumor Institute
University of Texas

Dr. M. Thomas Wagner, Executive Secretary
Assistant to the Chief
Radiation Bio-Effects Program
National Center for Radiological Health

Observers

Representing AEC:

Dr. Elmer B. Harvey
Medical Research Branch
Division of Biology and Medicine
Atomic Energy Commission

Representing AFWL:

Colonel Max M. Nold
Chief, Biophysics Branch
Research Division
Armed Forces Weapons Laboratory
Kirtland Air Force Base

Representing NIH:

Dr. Nathan W. Shock
Chief, Gerontology Branch
Baltimore City Hospitals

APPENDIX D

SEMINARS AND COLLOQUIA, 1967

Anthony N. Mikalis, Coordinator

SEMINARS

Speaker	Topic
Dr. Ernest L. Dobson Donner Laboratory University of California	Kinetics of Phagocytosis
Dr. Benjamin P. Sonnenblick Professor of Biology Rutgers, The State University	Some New and Some Old Problems in Radiation Biology
Dr. Shigeyoshi Odashima Research Member Sasaki Foundation, Sasaki Institute Tokyo, Japan	Conditioning Chemical Carcinogenesis
Dr. Arthur D. Bloom PSP Staff Officer Atomic Bomb Casualty Commission Nagasaki, Japan	Cytogenetic Studies in the Hiroshima and Nagasaki Survivors
Dr. Don L. Ridgeway Associate Professor of Biophysics Medical College of Virginia	Protein Structure in Solution
Dr. Warren R. Pistey Department of Pathology West Virginia Medical Center	Chromosomal Puffs - A Tool for the Study of Teratogenesis
Dr. Robert W. Miller Chief, Epidemiology Branch National Cancer Institute National Institutes of Health	Radiation and Other Factors Associated with an Increased Risk of Leukemia
Dr. Hoshiteru Nakamura Institute of Algological Research Hokkaido, Japan	The Role of Marine Plants in Radiation Protection
Dr. Francesco Mauro National Cancer Institute National Institutes of Health	Effects of Sulfur Mustard on Mammalian Cells Grown In Vitro

Speaker	Topic
Dr. Gustavus H. Klinck Chief, Endocrine Pathology Section Armed Forces Institute of Pathology Walter Reed Army Medical Center	In Thyroid: Light Cells, Hürthle Cells and Calcitonin
Dr. Arthur H. Wolff Chief, Radiation Bio-Effects Program National Center for Radiological Health	Observations on a Visit to the USSR

COLLOQUIA

Dr. Patricia Mishkin Physiology and Biophysics Laboratory Radiation Bio-Effects Program	Interference by X-Irradiation with the Induced Synthesis of Tryptophan Pyrrolase
Mr. Julius Barth Physiology and Biophysics Laboratory Radiation Bio-Effects Program	The Binding Properties of Radionuclides and Essential Cations by Clays in an Artificial Rumen-Simulated Gastrointestinal Procedure and in Lactating Goats
Dr. Howard R. Nankin Radiation Cytology Laboratory Radiation Bio-Effects Program	Satellite Association and Trisomy in Thyrotoxicosis
Dr. Wellington Moore, Jr. Radiation Cytology Laboratory Radiation Bio-Effects Program	Persistence of Chromosomal Aberrations in Chinese Hamster Thyroid Following Administration of ^{131}I
Mr. Charles J. Osicka Population Studies Program	Computerized Efficiency Determination of Data from a Liquid Scintillation Counter
Dr. DeWitt G. Hazzard Physiology and Biophysics Laboratory Radiation Bio-Effects Program	Verxite Flakes for In Vivo Binding of ^{134}Cs in Cows
Mr. Jesse Y. Harris Biometrics Support Unit Radiation Bio-Effects Program	Influence of Feed Additives on ^{131}I Excretion in Milk

Speaker	Topic
Mr. Jessy Y. Harris Biometrics Support Unit Radiation Bio-Effects Program	Effects of Feeding Resin on Levels of ^{85}Sr and ^{134}Cs in Milk
Mr. Harry Youmans, Jr. Chief, Radiation Physics Laboratory Radiation Bio-Effects Program	X-ray Injury to the Hand with Medical Report and Color Slides
Mr. Clayton Cisar Chief, Animal Care Unit Radiation Bio-Effects Program	Safe Handling of Animals Exposed to Radiation and Radioisotopes
Mr. Edward S. Weiss Deputy Chief, Population Studies Program	Thyroid Anomalies Among Children Presumably Exposed to Environmental Radioiodine
Dr. Norman C. Telles Chief, Radiation Oncology and Pathology Laboratory Radiation Bio-Effects Program	Radiation Carcinogenesis. A Review of Factors in Developing Criteria for Radiological Control
Dr. M. Thomas Wagner Office of the Chief Radiation Bio-Effects Program	The Effects of In Utero Radiation on Postnatal Enzyme Adaptation in the Beagle
Dr. Gordon L. Jessup Chief, Biometrics Support Unit Radiation Bio-Effects Program	Why Statistics?
Dr. Richard K. Fred Physiology and Biophysics Laboratory Radiation Bio-Effects Program	The Tissue Distribution of Intravenously Administered ^{14}C-Glucan
Mr. Richard P. Chiacchierini Biometrics Support Unit Radiation Bio-Effects Program	Experimental Error and Randomization
Mr. Barry Shemin Population Studies Program	The Validity of the Normal Approximation to the Difference between Two Poisson Random Variables

APPENDIX E

TECHNICAL AND PROFESSIONAL STAFF

Office of the Chief

Dr. Arthur H. Wolff, Chief
Dr. William A. Mills, Assistant Chief for Laboratory Operations
Dr. M. Thomas Wagner
Leo C. Cox
Irene A. Hardman
Donald M. Hodge

Biometrics Support Unit

Dr. Gordon L. Jessup, Chief
Richard P. Chiacchierini
Jesse Y. Harris

Animal Care Unit

Clayton F. Cisar, Chief

Radiation Oncology and Pathology Laboratory

Dr. Norman C. Telles, Chief
Stephen A. Carr
Joe R. Childress
Don W. Coble
Thomas R. Horton
Anthony N. Mikalis
Dr. Edward J. Pollock
Dr. Billy C. Ward
Elliott Willensky

Radiation Physics Laboratory

Harry D. Youmans, Chief
George E. Anderson
Ulrich H. Behling
John W. Laskey
Wah Lee
Jasper D. Manes
Frank C. Pendleton
Richard A. Tell

Physiology and Biophysics Laboratory

Dr. Moris L. Shore, Chief
Dr. Richard K. Fred
Dr. DeWitt G. Hazzard
David E. Janes
Anthony Lamanna
Dr. Patricia E. Sheppard
Thomas J. Withrow
Terrye G. Zaremba

Radionuclide Toxicology Laboratory

Dr. Jerry F. Stara, Chief
Dr. R. L. Blanchard
Alexander Martin
Dr. N. S. Nelson
Lawrence S. Rosenstein
Dr. R. G. Wolfangel

Collaborative Radiological Health Laboratory

Dr. R. John Garner, Director
Dr. Ezra Berman
Dr. Audrey K. Davis
Dr. Paul D. Lambert
Dr. Robert D. Phemister
Dr. James N. Shively
Dr. Barry P. Stuart
Dr. Robert W. Thomassen

Radiation Cytology Laboratory

Dr. William M. Leach, Chief
Dr. Julian G. Abuelo
Steven Brecher
Darilyn A. Dolimpio
Dinnemaud J. Ellingson
Dorothy E. Moore
Dr. Howard R. Nankin
Dr. Kenneth T. S. Yao
Lillie J. Gillespie

☆U. S. GOVERNMENT PRINTING OFFICE : 1968 O - 296-712

END